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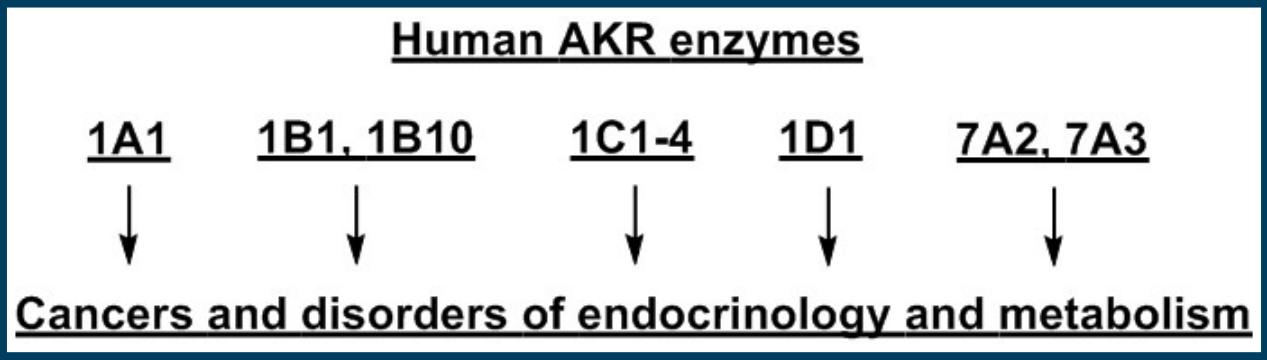

ALDO-KETO REDUCTASES AND ROLE IN HUMAN DISEASE

Topic Editor
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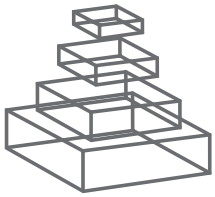

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ALDO-KETO REDUCTASES AND ROLE IN HUMAN DISEASE

Topic Editor:
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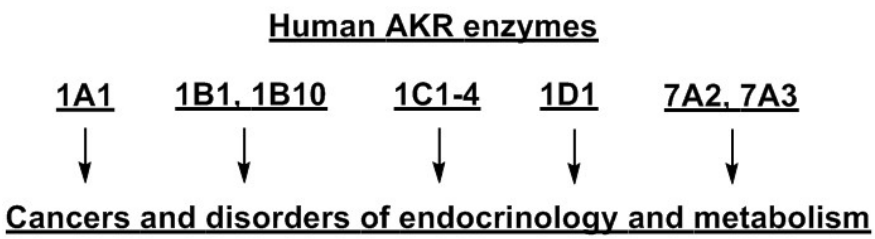

Human AKR enzymes and disease

Aldo-keto reductases (AKRs) are soluble NAD(P)(H) oxidoreductases that primarily catalyze the reduction of aldehydes and ketones to primary and secondary alcohols, respectively. The ten known human AKR enzymes can turnover a vast range of endogenous and exogenous substrates, including glucose, steroids, carcinogens, reactive aldehydes, and a variety of carbonyl-containing drugs. In recent years, the AKR enzymes have been implicated in a number of human diseases. As the rate limiting enzyme of the polyol pathway of glucose metabolism, AKR1B1 has long been known for its involvement in diabetic complications. Recent studies now suggest AKR1B1 play a key role in inflammatory diseases such as atherosclerosis, sepsis, asthma, uveitis, and colon cancer by mediating oxidative stress-induced inflammatory signals. AKRs are also implicated in the development and progression of many cancers as well as chemotherapeutic drug resistance. AKR1B1 and AKR1B10 were found to be overexpressed in tumors, such as liver, breast, and lung cancer. Several AKRs (AKR1A1, AKR1B10, and AKR1C1-3) are involved in tobacco-carcinogenesis because they activate polycyclic aromatic trans-dihydrodiols to yield reactive and redox active o-quinones, but they also catalyze the detoxication of nicotine derived nitrosamino ketones. In addition, AKR1C1-3 enzymes play a key role in the regulation of proliferative signaling in hormone dependent cancers. In recognition of the role of AKR in various diseases, significant efforts have been made in the development of specific/selective AKR1B1 and AKR1C3 inhibitors. The aim of this Research Topic forum is to celebrate the advancements seen in the AKR field via review papers and original articles as well as to promote future research in understanding the underlying mechanism(s) of the role of AKRs in human disease.

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EDITORIAL
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Editorial on research topic: aldo-keto reductases and role in human disease

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Research on Aldo-keto reductases (AKRs) over the years has revealed a remarkable diversity in the reactivity of these soluble NAD(P)(H) oxidoreductases. The AKR enzymes primarily catalyze the reduction of aldehydes and ketones to primary and secondary alcohols, respectively. The 10 known human AKR enzymes can turnover a vast range of endogenous and exogenous substrates, including glucose, steroids, carcinogens, reactive aldehydes, and a variety of carbonyl-containing drugs. These enzymes have been implicated in a number of human diseases, including cancer and disorders of endocrinology and metabolism. AKRs are involved in the development and progression of many cancers, as well as chemotherapeutic drug resistance. AKR1B1 and AKR1B10 are overexpressed in tumors, such as liver, breast, and lung cancer. Several AKRs (AKR1A1, AKR1B10, and AKR1C1-3) are involved in tobacco-carcinogenesis, but they also catalyze the detoxication of nicotine derived nitrosamino ketones. In addition, AKR1C1-3 enzymes play a key role in the regulation of proliferative signaling in hormone dependent cancers. AKR1B1 has long been known for its involvement in diabetic complications. Recent studies now suggest AKR1B enzymes play a key role in inflammatory diseases such as atherosclerosis, sepsis, asthma, uveitis, and colon cancer by mediating oxidative stress-induced inflammatory signals. In recognition of the role of AKR in various diseases, significant efforts have been made in the development of specific/selective AKR1B1 and AKR1C3 inhibitors. The aim of this Research Topic forum is to celebrate the advancements seen in the AKR field via review papers and original articles, as well as to promote future research in understanding the underlying mechanism(s) of the role of AKRs in human disease.

Eight review articles are included in this issue, and are grouped into disease areas (cancer, disorders of endrocrinology and metabolism) after the artile written by Chen and Zhang (2012) which describes the current understanding of the regulation of all human AKR members in relation to disease. The significance of the AKR enzymes for research in various cancers is discussed in four review articles. The article by Matsunaga et al. (2012) focuses on the role AKR1B10 in drug resistance of cancer cells and the development of AKR1B10 inhibitors to reverse chemoresistance. The article by Ruiz et al. (2012) describes the retinaldehyde reductase activity of AKRs in relation to proliferation and tumorigenesis. The paper from the Penning group (Zhang et al., 2012) summarizes the role of AKR in the metabolic activation and detoxication of polycyclic aromatic hydrocarbons and the relevance of phase II conjugation reactions to human lung carcinogenesis. Rižner (2012) provides a review on the role played by AKR1B and AKR1C members in the pathogenesis of endometrial and cervical cancers, as well as other uterine diseases. The aspect that members of AKRs can mediate the timing of parturition through metabolism of progesterone and prostaglandins is discussed by Byrns (2012). The role of AKR1B members in modification and production of signaling molecules is emphasized by Pastel et al. (2012) for a better understanding of the physiological and pathological role of AKR1B enzymes. The article by Tang et al. (2012) focuses on the increases in oxidative stress by AKR1B1 in pathogenesis of diabetic complications such as cardiovascular diseases.

Three Original Research Articles follow the review articles. The article by Fortier and coworkers (Bresson et al., 2012) reports on the prostaglandin *F* synthase activity of AKR1B1 in different models of living cells and tissues. This activity of AKR1B1 represents a novel target to regulate ischemic and inflammatory responses associated with several human pathologic conditions. The article by Laffin and Petrash (2012) examines the expression of AKR1B1 and AKR1B10 across all major human cancer types. Their findings point to the great potential of AKR1B1 inhibition as novel cancer therapeutics. Schulze et al. (2012) describe their studies on basal and regulatory promoter of *AKR1C3* in relation to prostate cancer.

As pointed out in the review article by Pastel et al. (2012) there is a "marked contrast between the abundance of enzymatic data and the small number of reports dedicated to functional studies in a physiological context." It is my hope that with an emphasis on the role of AKRs in human disease the articles included in this issue will benefit a broad audience and the researchers in the rapidly growing AKR filed.

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Regulation of aldo–keto reductases in human diseases

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The aldo–keto reductases (AKRs) are a superfamily of NAD(P)H-linked oxidoreductases, which reduce aldehydes and ketones to their respective primary and secondary alcohols. AKR enzymes are increasingly being recognized to play an important role in the transformation and detoxification of aldehydes and ketones generated during drug detoxification and xenobiotic metabolism. Many transcription factors have been identified to regulate the expression of human AKR genes, which could have profound effects on the metabolism of endogenous mediators and detoxication of chemical carcinogens. This review summarizes the current knowledge on AKR regulation by transcription factors and other mediators in human diseases.

Keywords: aldo–keto reductase, regulation, response element, transcription factor, human disease

INTRODUCTION

The aldo–keto reductases (AKRs) are a superfamily of NAD(P)H-linked oxidoreductases, which primarily catalyze the reduction of aldehydes and ketones to primary and secondary alcohols, respectively (Penning and Drury, 2007). AKRs are generally soluble, cytosolic monomeric (37 kDa) enzymes and are present in both prokaryotes and eukaryotes. These enzymes share a common protein fold, a (β/α)8 or a triosephosphate isomerase (TIM)-barrel, and have an active site in the C-terminal face (Jez et al., 1997a,b; Barski et al., 2008). Most AKRs use pyridine nucleotide as cofactors and catalyze simple oxidation–reduction reactions involved in the metabolism of sugar aldehydes, reactive lipid aldehydes, ketoprostaglandins, and ketosteroids.

Aldo–keto reductases consist of 15 families which have more than 100 members (Barski et al., 2008). There are 13 human AKR proteins that fall into 3 families AKR1, AKR6, and AKR7 (**Table 1**). The detailed information is available at http://www.med.upenn.edu/akr, an AKR superfamily homepage, maintained by Dr. T. Penning at the University of Pennsylvania. The human AKR enzymes include AKR1A1 (aldehyde reductase), AKR1B1 and AKR1B10 (aldose reductases), AKR1C1–C4 (hydroxysteroid dehydrogenases), AKR1D1 (steroid 5β-reductase), the AKR6 family (AKR6A3, A5, and A9; Kvβ proteins), and the AKR7 family (AKR7A2 and AKR7A3; aflatoxin aldehyde reductases). These human AKR enzymes can catalyze many metabolic oxidation–reduction reactions of numerous endogenous and exogenous substrates, including glucose, steroids, carcinogens, reactive aldehydes, and a variety of carbonyl-containing drugs. To date, the AKR enzymes have been implicated in a number of human diseases. It is known that AKR1A1, AKR1B10, and AKR1C1–C3 are involved in tobacco-induced carcinogenesis (Hsu et al., 2001; Penning, 2005; Jin and Penning, 2007; Zhang et al., 2008; Liu et al., 2009a). Inhibition of AKR1B1 is able to alleviate diabetic complications. Through mediating oxidative stress-induced inflammatory signals, AKR1B1 plays a role in inflammation-related diseases such as sepsis and colon cancer (Ramana, 2011). AKR1B1 and AKR1B10 regulate the development and progression of human liver, breast, and lung cancers through detoxifying reactive carbonyls, retinoic acid homeostatic regulation, and lipid metabolism (Liu et al., 2009a; Diez-Dacal et al., 2011). AKR1C1–C3 enzymes are involved in prostate and breast carcinogenesis (Penning and Byrns, 2009). Mutations in AKR1D1 gene in patients lead to neonatal cholestasis, hepatitis, and liver failure (Lemonde et al., 2003; Barski et al., 2008). AKR7A proteins protect liver from acetaminophen-induced hepatotoxicity by enhancing hepatocellular antioxidant defense (Ahmed et al., 2011). Because AKR enzymes are increasingly being recognized to play a role in various diseases, understanding the regulation of human AKR genes may help us develop novel therapeutic approaches. In this review, we summarize the regulation of AKRs by transcription factors, mediators, and pathological conditions.

THE REGULATION OF AKRs

Many transcription factors have been identified to regulate the expression of human AKR genes, which could have profound effects on the metabolism of endogenous mediators and detoxication of chemical carcinogens. Several classes of transcription enhancer elements have been identified in the upstream promoter region of AKR genes that include nuclear receptor response elements, AP-1 binding sites, the xenobiotic response elements (XRE), osmotic response elements (ORE), estrogen response elements (ERE), and antioxidant response elements (ARE; Ko et al., 1997; Burczynski et al., 1999; Lou et al., 2006; Penning and Drury, 2007).

AKR1A1

AKR1A1, a ubiquitously expressed enzyme, is a well-known cytosolic, NADPH-dependent and monomeric oxidoreductase. AKR1A1 participates in carbonyl reductions of chemotherapeutic drugs. Protein and mRNA levels of AKR1A1 in irinotecan-resistant LoVo cells are higher than in human colon adenocarcinoma LoVo cells (Peng et al., 2010). Recent studies suggest that overexpression

Table 1 | Regulation of human aldo–keto reductase.

Name	Enzyme	Previous symbols	Synonyms	Regulation
AKR1A1	Aldehyde reductase		ALR, DD3	hStaf/ZNF143, C/EBP, PPARγ, atorvastatin
AKR1B1	Aldose reductase	ALDR1	AR	Thyroid hormone, CREB, NAFT5, Nrf2, nitric oxide, EGF, TGFβ1, atorvastatin
AKR1B10	Aldose reductase	AKR1B11	AKR1B12, ARL-1, HIS, ARL1, HSI, ALDRLn	Mouse Akr1b7 is regulated by LXR, PXR, CAR, FXR
AKR1C1	Dihydrodiol dehydrogenase 1; 20-alpha (3-alpha)-hydroxysteroid dehydrogenase	DDH1	DDH, MBAB, DD1, HAKRC	Sp1, NF-Y, IL-1β
AKR1C2	Dihydrodiol dehydrogenase 2; bile acid binding protein; 3-alpha hydroxysteroid dehydrogenase, type III	DDH2	DD, BABP, DD2, HAKRD, MCDR2	IL-1β
AKR1C3	Aldo–keto reductase family 1, member C3 (3-alpha hydroxysteroid dehydrogenase, type II)	HSD17B5	KIAA0119, DDX, HAKRB	IL-6, cadmium, Nrf2
AKR1C4	Chlordecone reductase; 3-alpha hydroxysteroid dehydrogenase, type I; dihydrodiol dehydrogenase 4	CHDR	DD4, HAKRA, C11, 3-alpha-HSD, CDR, MGC22581	LXR
AKR1D1	Delta 4-3-ketosteroid-5-beta-reductase	SRD5B1		Estrogen
AKR6A3	Shaker channel β-subunit (Kvb1)		KCNAB1, KCNA1B, hKvBeta3, Kvb1.3, hKvb3	Bone morphogenic protein-2
AKR6A5	Shaker channel β-subunit (Kvb2)		KCNAB2, KCNA2B, HKvbeta2.1, HKvbeta2.2	
AKR6A9	Shaker channel β-subunit (Kvb1)		KCNAB3, KCNA3B	
AKR7A2	Aflatoxin aldehyde reductase		AFAR	Nrf2, acetaminophen
AKR7A3	Aflatoxin aldehyde reductase			Nrf2, acetaminophen

References and the regulation of AKRs by pathological conditions can be found in the text.

of AKR1A1 may protect lung epithelial cells against acute toxic effects of polycyclic aromatic hydrocarbon metabolites (Abedin et al., 2012).

Gel-shift assays and chromatin immunoprecipitation assays revealed that AKR1A1 is a target gene of transcription factors hStaf/ZNF143 and C/EBP homologous protein (**Table 1**; Barski et al., 2004; Myslinski et al., 2006). The β-hydroxy-β-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor atorvastatin suppresses the expression of AKR1A1 and AKR1B1 in human umbilical vein endothelial cells (HUVEC) but not in human proximal tubular epithelial cells (Ruf et al., 2009). Luciferase reporter assays showed that atorvastatin regulates AKR1A1 promoter activity through an ARE (Ruf et al., 2009). Grip et al. (2002) show that atorvastatin also activates PPARγ. The PPARγ binding sites are also found in the AKR1A1 promoter. Thus, atorvastatin may regulate AKR1A1 expression via direct or indirect regulation of AKR1A1 promoter activity.

AKR1B1 AND AKR1B10

AKR1B1, the most studied AKR family member, is the rate-limiting enzyme of the polyol pathway. AKR1B1 has long been known for its potential role in the development of diabetic complications by driving glucose flux through the polyol pathway (Liu et al., 2009a). Because this enzyme catalyzes the reduction of glucose to a sugar alcohol, many AKR1B1 inhibitors have been developed for the treatment of diabetic complications. Recent reports showed that AKR1B1 is also involved in inflammatory diseases such as atherosclerosis, sepsis, uveitis, and colon carcinogenesis through regulating oxidative stress-induced inflammatory signals (Ramana, 2011; Shoeb et al., 2011). The AKR1B1 and AKR1B10 have 71% identify in amino acid sequence. These two proteins show overlapped substrate specificity but significantly different tissue expression patterns. AKR1B1 is ubiquitously expressed whereas AKR1B10 is mainly expressed in liver, colon, small intestine, thymus, and adrenal gland. AKR1B10 was identified in 1998. Recent results suggest that AKR1B10 may be a tumor marker of several types of cancers. For example, Wang et al. (2010) reported that smoking can induce up-regulation of AKR1B10 in the airway epithelium, suggesting that increased AKR1B10 expression may be involved in lung carcinogenesis. Increased expression of AKR1B1 and AKR1B10 has been found in human liver, breast, and lung tumors. Thus these two proteins may play a key role in the development and progression of these types of cancers (Liu et al., 2009a; Kropotova et al., 2010).

The AKR1B1 gene is up-regulated in response to thyroid hormone (T3) treatment. Liao et al. (2009) reported that T3-bound thyroid hormone receptor (TR) induced AKR1B1 expression through a T3 response element; they also demonstrated that AKR1B1 overexpression in some types of hepatocellular carcinomas (HCCs) was TR-dependent, suggesting that the TR-AKR1B1

pathway might play a crucial role in the development of HCC. Lefrancois-Martinez et al. (2004) indicated that AKR1B1 expression in human adrenocortical cells may be regulated by the transcription factor cAMP-responsive element-binding protein (CREB). Yang et al. (2006) showed that the expression of AKR1B1 is tightly regulated by the transcription factor nuclear factor of activated T-cells 5 (NFAT5) through binding to OREs in the gene. Recently, the transcription factor nuclear factor-erythroid 2 related factor 2 (Nrf2), a key regulator in the adaptive response to oxidative stress, was shown to regulate the expression of AKR1B1, AKR1B10, and AKR1C1–C3 (Ebert et al., 2011; Nishinaka et al., 2011). Finally, several groups observed that the AKR1B1 expression is up-regulated in response to the treatment of nitric oxide (Seo et al., 2000), hydrogen peroxide, methylglyoxal (Yabe-Nishimura et al., 2003), epidermal growth factor, or transforming growth factor-β1 (Jiang et al., 2008).

The closest murine ortholog of human AKR1B10 is Akr1b7, which is also expressed in the liver, intestine, and adrenal gland. Murine Akr1b7 shares 89% amino acid homology with human AKR1B10. There are several nuclear receptor binding sites in the Akr1b7 promoter (**Figure 1**). Several groups have identified Akr1b7 as a direct target of nuclear receptors, such as liver X receptor (LXR; Volle et al., 2004), xenobiotic receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), and farnesoid X receptor (FXR; **Figure 1**). The finding that Akr1b7 is highly inducible by PXR and CAR (Liu et al., 2009b), is consistent with the role of Akr1b7 in xenobiotic metabolism and lipid peroxidation. Schmidt et al. (2011) showed that FXR induces Akr1b7 expression in the liver and intestine and that Akr1b7 plays a role in detoxification of specific bile acids. We found that hepatic and intestinal Akr1b7 is highly inducible by FXR and that Akr1b7 has striking effects on lowering blood glucose levels and reducing hepatic lipid accumulation in diabetic mice (Ge et al., 2011). These effects are associated with reduced expression of hepatic gluconeogenic genes and increased very low-density lipoprotein secretion. Our data suggest that AKR1b7 may be a therapeutic target for treatment of diabetes mellitus. It will be intriguing to investigate whether human AKR1B10 also regulates glucose and lipid metabolism.

AKR1C1–C4

AKR1C1–C4 share over 86% homology with each other. AKR1C1 is expressed in liver, kidney, and testis. AKR1C2 is mainly expressed in liver, prostate, and mammary gland. AKR1C3 is expressed in liver, brain, kidney, placenta, and testis. AKR1C4 is specifically expressed in liver. These enzymes are involved in the metabolism of steroid hormones (AKR1C1–AKR1C3), prostaglandins (AKR1C3), and bile acids (AKR1C4), and play important roles in the detoxification of drugs and xenobiotics (Ebert et al., 2011). AKR1C1–C3 enzymes may be involved in tobacco-induced prostate and breast carcinogenesis (Penning and Byrns, 2009). Increased expression of AKR1C1 has been detected in human diseases, such as endometriosis (Smuc et al., 2009; Hevir et al., 2011), androgen-independent prostate cancer (Stanbrough et al., 2006), and lung cancer (Hsu et al., 2001). Increased expression of AKR1C3 is found in leukemia (Mahadevan et al., 2006; Birtwistle et al., 2009), prostate cancer (Fung et al., 2006; Stanbrough et al., 2006), and breast cancer (Penning and Byrns, 2009).

AKR1C1 overexpression has been associated with drug-resistance in a variety of cancers (Deng et al., 2002, 2004; Chen et al., 2005; Hung et al., 2006). Some anticancer drugs may be metabolized by AKR1C1 due to the high similarity between the chemical structures of these drugs and AKR1C1 substrates (Selga et al., 2008). It has been proposed that increased AKR1C1 activity would detoxify reactive oxygen species induced by drugs such as cisplatin, and could lead to apoptosis-related development of drug-resistance. Selga et al. (2008) suggest that AKR1C1 gene expression is regulated by Sp1 transcription factor, and suppression of AKR1C1 by RNA interference (RNAi) improves the sensitivity to methotrexate, an antimetabolite drug for the chemotherapy of human malignancies, in methotrexate sensitive HT29 cells. The transcription factor, nuclear factor-Y (NF-Y) has also been reported to directly regulate the basal transcription of AKR1C1 in human ovarian, lung, and liver carcinoma cells (Pallai et al., 2010). Interleukin 1β (IL-1β), a major proinflammatory cytokine, can significantly induce the expression of both AKR1C1 and AKR1C2. Roberson et al. (2011) recently showed that this cytokine can facilitate local progesterone metabolism in a cell type critical for maintaining cervical structure through regulating

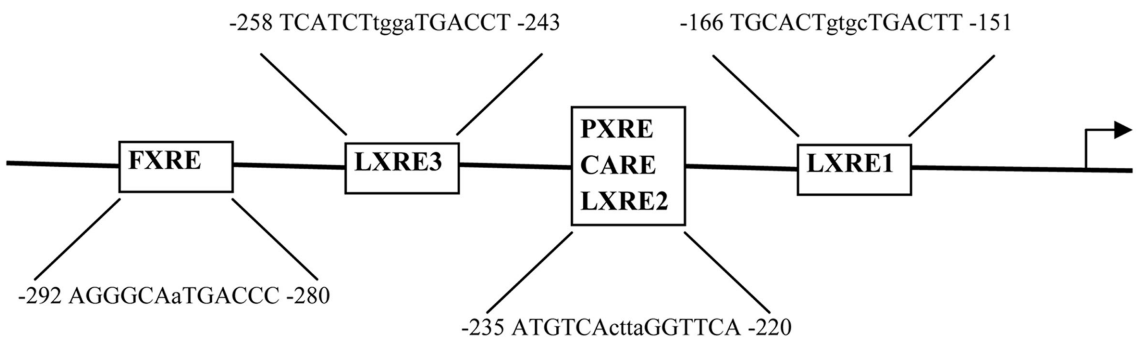

FIGURE 1 | Regulation of the Akr1b7 promoter activity by nuclear receptors. The DNA binding sites, including the FXR response element (FXRE), LXR response element (LXRE), PXR response element (PXRE), and CAR response element (CARE) are shown. There are three LXR binding sites (LXRE1-3) in the Akr1b7 promoter. In response to specific ligand treatments, the PXR/RXR, CAR/RXR, and LXR/RXR heterodimers bind to the same binding site (LXRE2) in the Akr1b7 promoter.

expression of AKR1C1 and AKR1C2. Chun et al. (2009) reported that IL-6 induces the expression of AKR1C3. Cadmium is a toxic metal. Occupational exposure to cadmium is related to the development of lung cancer. Lee et al. (2011) reported that cadmium induces AKR1C3 gene expression through activation of PI3K-related intracellular signaling pathways and Nrf2 activation. The significance in induction of AKR1C3 by IL-6 or cadmium remains to be explored.

It is well documented that liver X receptor (LXR), a nuclear receptor for oxysterols, plays an important role in the regulation of bile acid, lipid, and carbohydrate metabolism and inflammation. AKR1C4 has been demonstrated to be a direct target gene of LXR (Stayrook et al., 2008). Thus, AKR1C4 may play a role in LXR-regulated metabolism or inflammation.

OTHERS

AKR1D1 catalyzes the 5-β-reduction of bile acid intermediates and steroid hormones which carry a delta (4)-3-one structure. AKR1D1 is expressed in liver, colon, brain, and testis (Charbonneau and The, 2001; Jin et al., 2011). Deficiency of this enzyme may contribute to hepatic dysfunction (Lemonde et al., 2003; Gonzales et al., 2004; Clayton, 2011). Mutations in human AKR1D1 gene lead to neonatal cholestasis, hepatitis, and liver failure (Barski et al., 2008). In addition, AKR1D1 may play an important role in the degradative metabolism of sex hormones and in regulating multiple hormone-dependent processes. Mode and Rafter (1985) observed different levels of AKR1D1 in the liver of males and females. The ERE was found in the promoter of this gene, suggesting that AKR1D1 may be regulated by estrogen.

AKR6A3, A5, A9 are potassium voltage-gated channel beta-subunits, which are expressed in heart and brain. Fantozzi et al. (2006) suggested that bone morphogenetic protein-2 may suppress the expression of AKR6A3. AKR6A3 and AKR6A5 have been associated with epilepsy and impairment of learning and memory (Busolin et al., 2011; Wakasaya et al., 2011). Deletion of AKR6A5 is often detected in patients who have monosomy 1p36 deletion syndrome, which is characterized by learning disabilities (Perkowski and Murphy, 2011). By far, little is known about the regulation of AKR6A5 or A9.

AKR7A2 and AKR7A3 are aflatoxin dialdehyde reductases, which share the same chromosomal localization. These enzymes play important roles in bioactivation and biodetoxification (Barski et al., 2008). AKR7A2 is a ubiquitously expressed enzyme, whereas the expression of AKR7A3 is limited to the liver, kidney, colon, pancreas, stomach, endometrium, and adenocarcinoma. Previous studies suggested that AKR7A2 and A3, like other subgroups in the AKR superfamily, are transcriptionally regulated by oxidative stress-responsive transcription factor Nrf2. Ahmed et al. (2011) showed that AKR7A proteins are significantly upregulated in response to acetaminophen exposure and protect liver from acetaminophen-induced hepatotoxicity through enhancing hepatocellular antioxidant defense.

CONCLUSION

Aldo–keto reductase enzymes play an important role in the transformation and detoxification of aldehydes and ketones. They are involved in bile acid, lipid, carbohydrate, and xenobiotic metabolism, and in inflammation and carcinogenesis. By far, our understanding of the regulation of human AKRs remains limited. Further investigation of the regulation of human AKRs and AKR's functions under normal and pathological conditional will help develop novel therapeutic approaches for treatment of metabolic disorders, inflammation, cancer, and other relevant diseases.

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Aldo–keto reductase 1B10 and its role in proliferation capacity of drug-resistant cancers

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The human aldo–keto reductase AKR1B10, originally identified as an aldose reductase-like protein and human small intestine aldose reductase, is a cytosolic NADPH-dependent reductase that metabolizes a variety of endogenous compounds, such as aromatic and aliphatic aldehydes and dicarbonyl compounds, and some drug ketones. The enzyme is highly expressed in solid tumors of several tissues including lung and liver, and as such has received considerable interest as a relevant biomarker for the development of those tumors. In addition, AKR1B10 has been recently reported to be significantly up-regulated in some cancer cell lines (medulloblastoma D341 and colon cancer HT29) acquiring resistance toward chemotherapeutic agents (cyclophosphamide and mitomycin *c*), suggesting the validity of the enzyme as a chemoresistance marker. Although the detailed information on the AKR1B10-mediated mechanisms leading to the drug resistance process is not well understood so far, the enzyme has been proposed to be involved in functional regulations of cell proliferation and metabolism of drugs and endogenous lipids during the development of chemoresistance. This article reviews the current literature focusing mainly on expression profile and roles of AKR1B10 in the drug resistance of cancer cells. Recent developments of AKR1B10 inhibitors and their usefulness in restoring sensitivity to anticancer drugs are also reviewed.

Keywords: aldo–keto reductase 1B10, chemotherapy, resistance, proliferation

INTRODUCTION

The aldo–keto reductase (AKR) superfamily is a rapidly growing group of NAD(P)(H)-dependent oxidoreductases that metabolize carbohydrates, steroids, prostaglandins, and other endogenous aldehydes and ketones, as well as xenobiotic compounds (http://www.med.upenn.edu/akr/). Members of this superfamily are classified into 15 families, and each family is subdivided into several subfamilies based on their amino acid sequence similarities. For examples, human aldehyde reductase and aldose reductase are named AKR1A1 and AKR1B1, respectively. Four human hydroxysteroid dehydrogenases (HSDs), 20α-HSD, type 3 3α-HSD, type 2 3α-HSD/type 5 17β-HSD, and type 1 3α-HSD, are named AKR1C1, AKR1C2, AKR1C3, and AKR1C4, respectively, and are also collectively called AKR1C isoforms. The structural, biochemical, and physiological features of 14 human AKRs have been reviewed (Matsunaga et al., 2006; Jin and Penning, 2007; Barski et al., 2008). Among them, AKR1B1 is the most extensively studied, because it is the first enzyme of the polyol pathway metabolizing glucose into sorbitol and implicated in the chronic complications of diabetes such as retinopathy, neuropathy, and nephropathy (Yabe-Nishimura, 1998). In addition, human aldose reductase-like protein AKR1B10 has been recently regarded not only as a potential diagnostic and/or prognostic marker in carcinomas and serum (Fukumoto et al., 2005; Luo et al., 2011), but also as a therapeutic target for the prevention and treatment of several types of cancer (Liu et al., 2009a). Since AKR1B10 was originally identified as an up-regulated protein in hepatocellular carcinomas (Cao et al., 1998), its overexpression is observed in other types of cancer, and its involvement in the development of resistance toward anticancer agents has been suggested. The purpose of this article is to review recent advances on roles of AKR1B10 in cancer cells and their drug resistance. In addition, we provide an update on the usefulness of AKR1B10 inhibitors in restoring sensitivity to anticancer drugs.

PROPERTIES OF AKR1B10 AND ITS ROLE IN CANCER CELLS

AKR1B10 is a 36-kDa cytosolic reductase that is similar to AKR1B1 in both amino acid sequence identity (71%) and tertiary structure with the $(\alpha/\beta)_8$ barrel topology (Gallego et al., 2007). Like AKR1B1, AKR1B10 reduces a variety of aromatic and aliphatic aldehydes, dicarbonyl compounds, and some drug ketones using NADPH as the coenzyme (Cao et al., 1998; Martin et al., 2006; Spite et al., 2007; Baba et al., 2009; Endo et al., 2009; Zhong et al., 2009), and the reaction catalyzed by the enzyme follows an ordered bi bi mechanism with the binding of coenzyme to the free enzyme (Endo et al., 2009). However, AKR1B10 differs from AKR1B1 not only in its inability to reduce glucose and xylose (Cao et al., 1998; Crosas et al., 2003) and prostaglandin H_2 (Kabututu et al., 2009), but also in its high catalytic efficiency for retinals (Crosas et al., 2003; Gallego et al., 2006), isoprenyl aldehydes (farnesal and geranylgeranial; Endo et al., 2009), and cytotoxic aldehydes (acrolein and 4-hydroxy-2-nonenal; Shen et al., 2011).

AKR1B10 reduces all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal to their corresponding retinols with low K_m (0.6–13 μM) and high k_{cat}/K_m values (5.8–45 min^{-1} μM^{-1}; Crosas et al., 2003; Gallego et al., 2006). Retinoids play a pivotal role in proliferation, differentiation, and morphogenesis of many cell types through their binding of retinoic acids to retinoic acid receptors or retinoid X receptors (Bushue and Wan, 2010; Tang and Gudas, 2011). Retinoic acids are signaling molecules for inducing cell differentiation (Tang and Gudas, 2011), and are formed by sequential oxidation of retinols (**Figure 1A**). AKR1B10 regulates the retinoic acid homeostasis by decreasing the cellular levels of retinoic acids through efficiently reducing retinals into retinols, and is thus thought to participate in tumor development (Crosas et al., 2003; Gallego et al., 2006, 2007; Ruiz et al., 2009).

AKR1B10 also efficiently reduces farnesal and geranylgeranial into their alcohols (farnesol and geranylgeraniol) with low K_m (2.3 and 0.9 μM, respectively) and high k_{cat}/K_m values (13 and 11 min^{-1} μM^{-1}, respectively; Endo et al., 2009). Similar to the retinoid metabolism, the isoprenyl aldehydes are the intermediates in the metabolism of farnesol and geranylgeraniol into their carboxylic acids (farnesoic acid and geranylgeranoic acid; Endo et al., 2011), as illustrated in **Figure 1B**. Farnesol and geranylgeraniol are phosphorylated to their pyrophosphates, which are used for prenylation of cellular proteins including small G-proteins that are responsible for cell proliferation through activation of mitogen-activated protein (MAP) kinase cascade (Park and Beese, 1997; Sinensky, 2000). The reduction of the isoprenyl aldehydes by AKR1B10 increases the cellular levels of farnesol and geranylgeraniol, which promote protein prenylation. AKR1B10 may also participate in the tumor development by regulating isoprenoid metabolism.

In addition to its role in detoxification of cytotoxic carbonyl compounds (Shen et al., 2011), AKR1B10 promotes fatty acid and lipid synthesis by blocking the ubiquitin-dependent degradation of acetyl-CoA carboxylase α, a key enzyme in fatty acid synthesis, suggesting that the enzyme also promotes cell growth and survival through modulating lipid synthesis and membrane function (Ma et al., 2008; Wang et al., 2009). Although the following four roles of AKR1B10 in cancer cells have been proposed: (a) detoxification of cytotoxic carbonyl compounds, (b) promoting fatty acid and lipid synthesis, (c) reducing farnesal and geranylgeranial into their alcohols, and (d) reducing all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal to their corresponding retinols, further studies are needed to elucidate which is the key role in cellular growth and/or survival of each types of cancer described below.

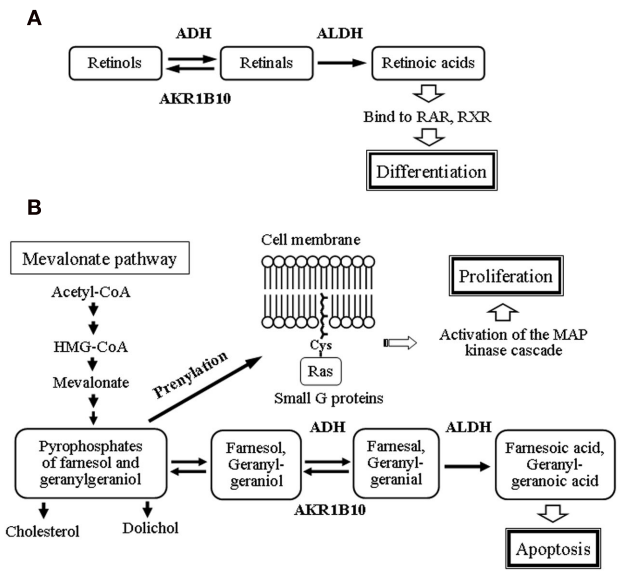


FIGURE 1 | Metabolism of retinoids (A) and isoprenoids (B). (A) all-*trans*-Retinol, 13-*cis*-retinol, and 9-*cis*-retinol are oxidized to the corresponding retinoic acids through retinals by alcohol dehydrogenases (ADH) and aldehyde dehydrogenases (ALDH). Retinoic acids regulate cell function including differentiation through binding to retinoic acid receptor (RAR) and retinoid X receptor (RXR). AKR1B10 effectively reduces retinals into retinols, and is considered to promote cell proliferation by inhibiting the retinoic acid formation. **(B)** Farnesyl pyrophosphate, an isoprenoid intermediate of the mevalonate pathway, is converted by its dephosphorylation into farnesol, which is further oxidized into farnesal and farnesoic acid by ADH and ALDH. Geranylgeranyl pyrophosphate is similarly metabolized. The two isoprenyl pyrophosphates are used in the prenylation of small G-proteins such as Ras, leading to activation of MAP kinase cascade and resultant increase in proliferation potential of cells. AKR1B10 efficiently reduces farnesal and geranylgeranial to farnesol and geranylgeraniol, respectively, which are phosphorylated into the isoprenyl pyrophosphates. Up-regulation of AKR1B10 in carcinomas and chemoresistant cancer cells may increase the cellular levels of the two isoprenyl alcohols by reducing back their aldehyde metabolites, and then promote proliferation of the cell.

CANCER-ASSOCIATED OVEREXPRESSION OF AKR1B10

In contrast to ubiquitous distribution of AKR1B1 (Grimshaw and Mathur, 1989), AKR1B10 is highly expressed in human gastrointestinal tract and adrenal gland, but its expression levels in other tissues are low (Cao et al., 1998; Hyndman and Flynn, 1998; Fukumoto et al., 2005). Of the two AKRs, AKR1B10 is significantly up-regulated in large and small airways of healthy smokers, while it is less detected in those of healthy non-smokers (Pierrou et al., 2007; Zhang et al., 2008; Wang et al., 2010). The up-regulation of AKR1B10 is possibly due to components in tobacco smoke (Nagaraj et al., 2006; Penning and Lerman, 2008). In addition, the overexpression of AKR1B10 is reported in the following malignant tumors. In lung cancer, AKR1B10, but not AKR1B1, is overexpressed, and the incidence of its overexpression differs depending on cancer types. Fukumoto et al. (2005) first reported the overexpression of AKR1B10 in most cases of squamous cell lung carcinomas and 29% of adenocarcinomas, both of which are also associated with smoking. The finding is confirmed by subsequent studies, in which AKR1B10 overexpression is more frequently detected in smokers with squamous cell lung cancer than in non-smokers with the cancer or smokers with adenocarcinomas (Woenckhaus et al., 2006; Kim et al., 2007; Li et al., 2008; Kang et al., 2011). Hence, AKR1B10 is considered as a potential diagnostic marker of smoking-associated squamous cell lung carcinoma. AKR1B10 activates pro-carcinogenic polycyclic aromatic hydrocarbons in tobacco smoke by exhibiting dihydrodiol dehydrogenase activity (Quinn et al., 2008), suggesting a possibility that this enzyme is involved in carcinogenesis of the smoking-associated

lung carcinoma. In malignant liver tumors, both levels of AKR1B1 and AKR1B10 are increased, but the overexpression frequency of AKR1B10 is higher than that of AKR1B1 (Cao et al., 1998; Scuric et al., 1998; Lee et al., 2001; Teramoto et al., 2008; Satow et al., 2010). Recent clinicopathological studies show that the expression of AKR1B10 differs depending on stages of hepatocellular carcinomas: The enzyme is significantly overexpressed in lower tumor stages with underlying cirrhosis or viral hepatitis, whereas it is down-regulated in advanced tumor stages with low-grade of differentiation (Heringlake et al., 2010; Schmitz et al., 2011). Thus, AKR1B10 may be a valuable biomarker of differentiation and proliferation of liver tumor, and play a role in early phases of hepatocarcinogenesis. The up-regulation of AKR1B10 is also reported in uterine carcinomas (Yoshitake et al., 2007), cholangiocarcinomas (Heringlake et al., 2010), early stage gastric tumor (Lee et al., 2010), and esophageal carcinogenesis (Breton et al., 2008).

The cancer-associated overexpression of AKR1B10 probably results from an adaptive response to oncogenic processes, but little is known about its gene regulation. Liu et al. (2009b) characterized the promoter region of the AKR1B10 gene, which includes multiple putative oncogenic and tumor suppressor protein binding sites, including the transcriptional factors c-Ets-1 and C/EBP, the repressor protein p53, and the AP-1 oncogene. Nishinaka et al. (2011) identified several putative regulatory motifs, such as AP-1, NF-κB, and antioxidant response element, in a −3282 bp of the 5′-flanking region and 5′-untranslation region of AKR1B10 gene, and also suggest a possibility that a complex polymorphic microsatellite in this region is implicated in the enzyme induction in response to certain stimuli such as carcinogens. Further investigations on the regulatory mechanisms of AKR1B10 gene expression are needed to elucidate the significance of its induction in cancer cells.

ALTERATION IN EXPRESSION LEVELS OF AKRs IN CHEMORESISTANT CELLS

Chemotherapy is commonly utilized as a therapeutic approach for many cancers. However, one of major problems occurring during the therapy is the intrinsic or drug resistance of the cancer cells, which leads to malignant transformation and metastasis of tumors. Previous studies proposed several candidate molecules that play key roles in the mechanisms of chemoresistance. One of the most recognized candidates is an efflux transporter P-glycoprotein. As P-glycoprotein is called multidrug resistance protein, its aberrant expression decreases the metabolic clearance of the drugs through shortening its residence time in the cells (Higgins, 2007). The chemoresistance of the cancer cells is also provoked by high expression of an inhibitor of apoptosis (IAP) family members, such as c-IAP1 (Yang and Li, 2000; Notarbartolo et al., 2004), which is considered to inhibit apoptotic cell death elicited by some chemotherapeutics, such as cisplatin and doxorubicin, through directly interacting with caspases and then abolishing their pro-apoptotic actions (Vaziri et al., 2003; Tirrò et al., 2006). Other resistance-related factors are growth factor receptors and the ubiquitin–proteasome system. Binding of ligands to fibroblast growth factor receptor-1 and -3 activates both signalings of MAP kinase and phosphoinositide 3-kinase (Roidl et al., 2009; Tomlinson et al., 2012). Therefore, overexpression of the growth factor receptors may enhance capacities of resistant cells to proliferate, and protect from fatal damage induced by anticancer drugs. The ubiquitin–proteasome system, a large multi-subunit complex that cleaves damaged and misfolded proteins, is thought to be activated with cancer chemoresistance. The involvement of proteasomes in the chemoresistance is obvious from data that treatment with proteasome inhibitors MG132 and bortezomib induces multidrug resistance-associated protein 2 in human colon cancers (Loeffler-Ragg et al., 2009; Ebert et al., 2011). In addition, the proteasome modulates the expression of transcription factors, such as NF-κB, p53, c-Jun, and c-Fos, that participate in the mechanisms of cell proliferation and differentiation (Cory and Cory, 2002; Li et al., 2007). Moreover, the ubiquitin–proteasome system appears to regulate cellular susceptibility to death by controlling the balance between pro-apoptotic caspases and anti-apoptotic Bcl and IAPs (Almond and Cohen, 2002).

A growing body of evidence implicates some members of the AKR superfamily as new candidates that are involved in acquisition of chemoresistance. Among the members, AKR1B1, AKR1B10, and three AKR1C isoforms (AKR1C1, AKR1C2, and AKR1C3) are reported to be up-regulated in several cancers exposed to anticancer drugs (**Table 1**). Although clinical studies are few, Chen et al. (2005) showed that the three AKR1C isoforms are up-regulated in ovarian cancers, in which their overexpression extents in cases resistant to cisplatin are much higher than those in cisplatin-sensitive ones. Ueda et al. (2006) also reported that AKR1C1/1C2 are highly expressed in uterine cervical cancer with papillomavirus infection, and suggests that their high expression is closely associated with drug resistance to cisplatin and anthracyclines.

Studies using the resistant phenotypes of cancer cells prepared by exposure to anticancer drugs provided some informative results concerning the relationship of the AKR expression with the drug resistance. The overexpression of the three AKR1C isoforms due to cisplatin resistance is observed in resistant phenotypes of ovarian cancer cells (Deng et al., 2002; Chen et al., 2005, 2008). In the resistance to anthracyclines, resistance of breast tumor cells to doxorubicin and epirubicin increases the expression levels of AKR1C2 and AKR1C3 (Veitch et al., 2009), and daunorubicin-resistant variant cells of stomach cancer highly express the mRNAs for AKR1B1 and AKR1C2 (Ax et al., 2000). In the resistance to other agents, magnitude of resistance to cyclophosphamide (in medulloblastoma) and methotrexate (in colon cancer cells) is correlated with up-regulation of AKR1B10 and AKR1C1, respectively (Bacolod et al., 2008; Selga et al., 2008). We recently found high expression of AKR1B10 in mitomycin *c*-resistant phenotypes of colon cancer HT29 cells (Matsunaga et al., 2011). In addition to the studies using the resistant phenotypes, cell-based experimental techniques of transgenesis and small RNA interference elucidate crucial roles of the five enzymes in chemoresistance of cells derived from various cancers, as listed in **Table 1**.

Gain of resistance to platinum drugs, cisplatin, and carboplatin, increases the expression levels of AKR1C1 and AKR1C2 in cells derived from several cancers of ovary, cervix, lung, and germ cell (Deng et al., 2004). However, the effect of resistance to platinum drugs on the expression of AKRs in colon cancer has

Table 1 | Expression of AKRs in chemoresistant cancer cells.

Cancer	Drug	Up-regulated enzyme	References
CLINICAL INVESTIGATIONS			
Ovarian cancer	Cisplatin	AKR1C1, AKR1C2, AKR1C3	Chen et al. (2005)
Uterine cervical cancer	Cisplatin	AKR1C1, AKR1C2	Ueda et al. (2006)
	Doxorubicin		
INVESTIGATIONS USING RESISTANT PHENOTYPES			
Ovarian cancer	Cisplatin	AKR1C1, AKR1C2, AKR1C3	Deng et al. (2002)
			Chen et al. (2008)
Breast cancer	Doxorubicin	AKR1C2, AKR1C3	Veitch et al. (2009)
	Epirubicin		
Stomach cancer	Daunorubicin	AKR1B1, AKR1C2	Ax et al. (2000)
Medulloblastoma	Cyclophosphamide	AKR1B10	Bacolod et al. (2008)
Colon cancer	Methotrexate	AKR1C1	Selga et al. (2008)
	Mitomycin *c*	AKR1B10	Matsunaga et al. (2011)
INVESTIGATIONS BY TECHNIQUES OF GENE OVEREXPRESSION AND SILENCING			
Ovarian, cervical, and lung cancers	Cisplatin	AKR1C1, AKR1C2	Deng et al. (2004)
	Carboplatin		
Lung cancer	Cisplatin	AKR1C1, AKR1C2	Hung et al. (2006)
	Adriamycin		Wang et al. (2007)
Cutaneous cancer	Bleomycin	AKR1C1, AKR1C2	Chow et al. (2006)
Pancreas cancer	Daunorubicin	AKR1B1	Plebuch et al. (2007)
Breast cancer	Doxorubicin	AKR1C1	Loignon et al. (2009)
	Paclitaxel		

not been studied. To date, oxaliplatin (L-OHP), a third generation platinum analog, is used for treatment of colon cancer, especially the metastatic colorectal cancer. Our recent data on the effect of L-OHP on the expression of AKRs in colon cancer HT29 cells are shown below. We established low-grade (HT29/L-OHP2) and high-grade (HT29/L-OHP20) resistant phenotypes, which show resistance to the drug concentrations of 2 and 20 μM, respectively. The parental cells express AKR1C1, AKR1C2, AKR1C3, and AKR1B10, but not AKR1B1, which is not detected even in the resistant phenotypes. In the low-grade resistant cells, only AKR1B10 is highly expressed in contrast to no apparent changes in the levels of AKR1C1/1C2 and AKR1C3 (**Figure 2**). In contrast, the levels of all AKRs are remarkably low in the high-grade resistant cells. Since the HT29/L-OHP20 cells show 10-fold higher 26S proteasome activity than the control cells, it is a persuasive argument that the facilitation of the proteasome function is involved in the down-regulation of the AKRs except for AKR1B1.

A well accepted mechanism for induction of AKR1B10, AKR1C1, AKR1C2, and AKR1C3 is a nuclear factor erythroid 2-related factor 2 (Nrf2)-Kelch-like ECH-associated protein 1 (Keap1) system (Penning and Lerman, 2008; MacLeod et al., 2009; Nishinaka et al., 2011; Agyeman et al., 2012). Under normal conditions, the transcription factor Nrf2 tightly binds to Keap1 in the cytosol and is degraded by the ubiquitin–proteasome system (Lau et al., 2008). Nrf2 is dissociated from Keap1 in response to various stimuli including reactive oxygen species (ROS; De Vries et al., 2008) and electrophiles (Levonen et al., 2004), translocates into the nuclei, and binds to antioxidant response element in genes to transcriptionally induce proteins including the above AKRs. Recently,

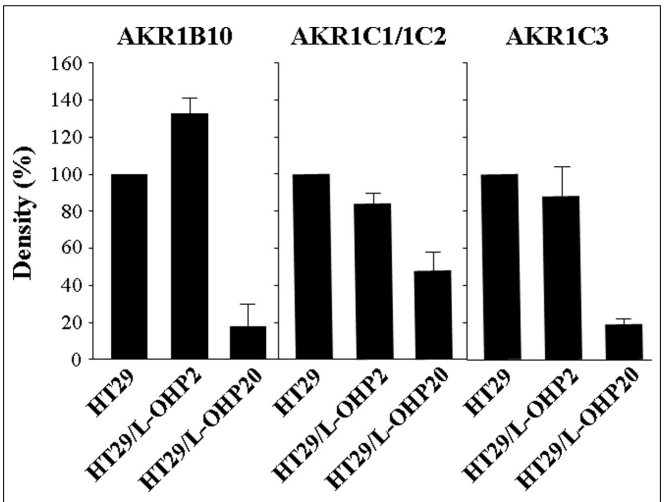


FIGURE 2 | Expression levels of AKRs in parental and L-OHP-resistant HT29 cells. The cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics. The two L-OHP-resistant cells HT29/L-OHP2 and HT29/L-OHP20 were prepared by exposing of the parental cells to incremental stepwise concentrations (0.05–2 and 20 μM, respectively) of L-OHP. The amounts of AKR1B10, AKR1C1/1C2, and AKR1C3 in the cell extracts were analyzed by Western blotting using the antibodies against the enzymes (Matsunaga et al., 2011). The density of the immunoreactive band is normalized to that in the parental cells (taken as 100%), and expressed as the mean ± SD of three independent experiments. AKR1C1/1C2: total amount of AKR1C1 and AKR1C2 detected by the antibody cross-react with the two enzymes.

Jiang et al. (2010) showed a relationship between abnormal activation of Nrf2 in type II endometrial cancer and the development of chemoresistance. This is supported by findings that the overexpression and knockdown of Nrf2 result in increase and decrease, respectively, in chemoresistance of some types of cancer cells (Cho et al., 2008; Wang et al., 2008; Shim et al., 2009). Recent studies proposed two theories as the reasonable mechanistic base for hyperactivation of Nrf2 in the cancer cells resistant to chemotherapy. Loignon et al. (2009) showed that the Nrf2 hyperactivation is due to down-regulation of Cullin 3 ubiquitin E3 ligase, an adaptor protein that specifically targets Nrf2 for degradation in the ubiquitin–proteasome system, finally leading to sensitization to doxorubicin and paclitaxel. In addition, mutations in Nrf2/Keap1 complex, especially in Keap1, to lose the Nrf2–Keap1 interaction are identified in cancer cell lines showing chemoresistance (Singh et al., 2006; Shibata et al., 2008). Thus, the Nrf2 activation is considered as a crucial event in the development of chemoresistance. In contrast, our data, together with studies listed in **Table 1**, clearly indicate that expression patterns of the Nrf2-related genes AKR1B10, AKR1C1/1C2, and AKR1C3 differ depending on both cell types and anticancer drugs involved in resistance induction. Hence, the different expression patterns of AKRs in chemoresistant cancers may result from complex mechanisms that regulate the synthesis and degradation of AKR proteins.

POSSIBLE ROLES OF AKR1B10 IN CHEMORESISTANT CANCER CELLS

METABOLISM OF ANTICANCER DRUGS

AKR1B10 converts daunorubicin and idarubicin into less toxic forms daunorubicinol and idarubicinol, respectively, by reducing C13 ketone groups, with K_m values ranging from 0.4 to 10 mM (Martin et al., 2006; Balendiran, 2009; Balendiran et al., 2009; Bains et al., 2010; Zhong et al., 2011). The enzyme also reduces doxorubicin and epirubicin albeit at lower rates (Bains et al., 2010; Zhong et al., 2011). The artificial overexpression and inhibition of AKR1B10 in 293 T cells result in decrease and increase, respectively, in sensitivity to daunorubicin and idarubicin (Zhong et al., 2011). These findings suggest that the AKR1B10 up-regulation is responsible for the resistance to the anthracyclines. In addition, AKR1B10 is suggested to be involved in acquisition of the resistance of medulloblastoma to cyclophosphamide through a deactivation of a reactive cyclophosphamide metabolite aldophosphamide (Bacolod et al., 2008). Furthermore, we recently found a significant increase in NADPH-linked reductase activity toward mitomycin *c* in the drug-resistant HT29 cells (unpublished data), in which AKR1B10 is significantly overexpressed (Matsunaga et al., 2011). This may imply that the involvement of AKR1B10 in the mitomycin *c* resistance is in part due to its ability to metabolize this drug.

CELL PROLIFERATION

AKR1B10 is suggested to enhance cancer cell proliferation (Zu et al., 2009), and its gene silencing suppresses growth of human colon cancer HCT-8 cells (Yan et al., 2007). As shown in **Figure 3**, overexpression of AKR1B10 in human leukemic monocyte lymphoma U937 cells significantly increases the cell proliferation, which is abrogated by the addition of a potent AKR1B10 inhibitor (*Z*)-2-(4-methoxyphenylimino)-7-hydroxy-*N*-(pyridine-2-yl)-2*H*-chromene-3-carboxamide (PHPC; Endo et al., 2010a). These findings predict the involvement of AKR1B10 in proliferative potential of many cancer cells, which may be also related to its overexpression in several cancer lesions (as mentioned in Cancer-Associated Overexpression of AKR1B10).

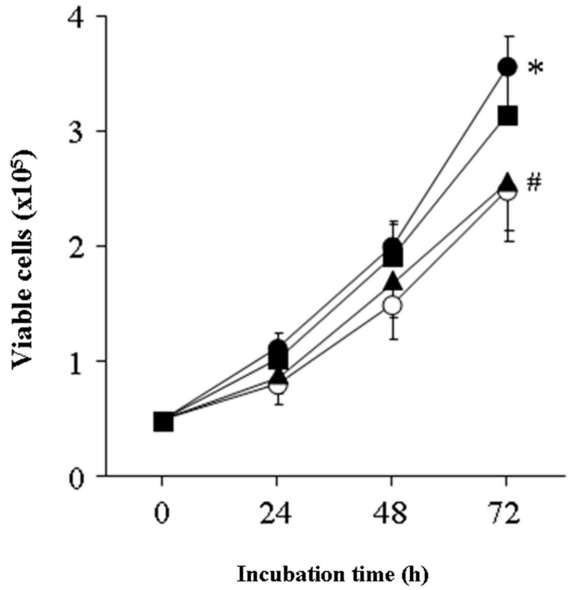


FIGURE 3 | Overexpression of AKR1B10 elevates proliferation potential in U937 cells. U937 cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and antibiotics, and transfected with the expression vector harboring the AKR1B10 cDNA as described previously (Endo et al., 2009). The cell expressed AKR1B10, which was not detected in the control cells transfected with the empty vector. The cells were seeded at a density of 5×10^4 cells/well into a 24-well multiplate, and cultured for 24, 48, or 72 h after addition of 0 (●), 5 (■), or 20 μM (▲) of the AKR1B10 inhibitor, PHPC. The number of viable cells was evaluated by the trypan blue-dye exclusion method, and is expressed as the mean ± SD of three independent experiments. Since no significant effect of PHPC on growth of the control cells was observed, only the proliferation of the control cells without PHPC (○) is depicted. *Significant difference ($p < 0.05$) for the overexpressing cells without PHPC (●) from the control cells (○). #Significant difference for the overexpressing cells with 20 μM PHPC (▲) from those without the inhibitor (●).

The acquisition of multidrug resistance in a variety of cancer cells has been shown to decrease tumorigenicity and prolong doubling time due to a G_2/M phase elongation (O'Loughlin et al., 2000; De Angelis et al., 2006). However, to our knowledge, there have been no reports on a relationship between AKR1B10 expression and proliferative potential in chemoresistant cancer cells. When the two L-OHP-resistant and the parental HT29 cells that are described in **Figure 2** are compared with respect to proliferation rate and DNA replication potential, there is a strong correlation between the two potentials and AKR1B10 level expressed in the cells. As shown in **Figure 4A**, the parental cells exhibit more rapid growth than HT29/L-OHP20 cells (with much lower level of AKR1B10 than the parental cells). The involvement of AKR1B10 in the proliferative potential is evidenced by significant

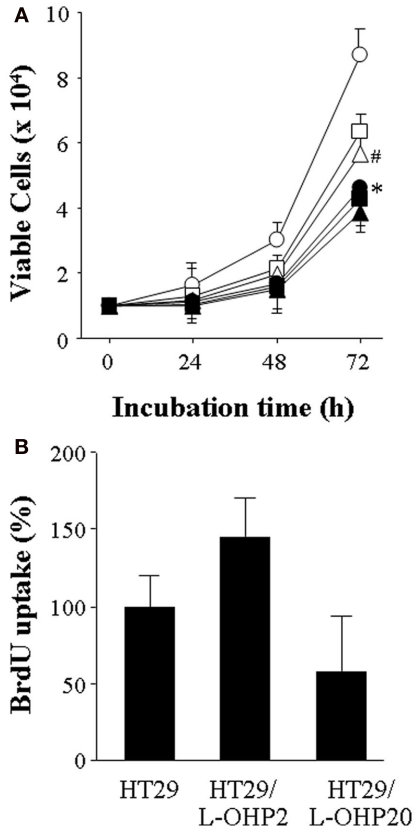


FIGURE 4 | Alteration in proliferation (A) and DNA replication potential (B) of HT29 cells by gain of resistance to L-OHP. (A) HT29/L-OHP20 (closed symbols) and the parental cells (open symbols) were cultured in the presence of 0 (circles), 5 (squares), or 20 μM PHPC (triangles), and their viable cell numbers were evaluated as described in **Figure 2**. Significant difference ($p < 0.05$) for HT29/L-OHP20 cells with 0 μM (●)* or the parental cells with 20 μM PHPC (Δ)# from the parental cells without PHPC (○). **(B)** Uptake of 5-bromo-2′-deoxyuridine (BrdU) into DNAs in the parental (HT29) and resistant (HT29/L-OHP2 and HT29/L-OHP20) cells was measured using a cell proliferation ELISA biotrak system (GE healthcare, Buckinghamshire, UK). Value is normalized to that in the parental cells (taken as 100%), and expressed as the mean ± SD of three independent experiments.

suppression of the proliferation of the parental cells, but not HT29/L-OHP20 cells, by the AKR1B10 inhibitor PHPC. In the DNA replication potential (**Figure 4B**), the order of 5-bromo-2′-deoxyuridine (BrdU) uptake is HT29/L-OHP2 cells > the parental cells > HT29/L-OHP20 cells, which also correlates with the expression levels of AKR1B10 shown in **Figure 2**. Flow cytometry analysis of the resistant cells reveals that shortening of G_1 phase and G_2/M phase elongation are associated with the magnitude of the L-OHP resistance (**Table 2**), but not with the AKR1B10 expression. Thus, AKR1B10 may participate in the proliferative mechanism of L-OHP-resistant colon cancer cells, although it affects the cell cycle less.

As mentioned in its roles in cancer cells, AKR1B10 is thought to participate in tumor development by detoxifying cytotoxic aldehydes and modulating the retinoic acid homeostasis, isoprenoid metabolism, and lipid metabolism. In contrast, little is known

Table 2 | Cell cycle of L-OHP-resistant HT29 cells.

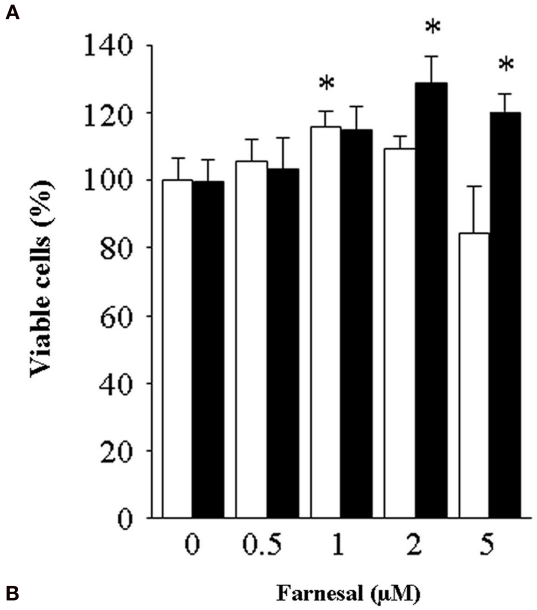
Cells	Phase (%)		
	G_1	S	G_2/M
HT29	77.2	3.9	18.9
HT29/L-OHP2	58.3	14.2	27.5
HT29/L-OHP20	34.0	14.8	51.2

The parental HT29 cells and L-OHP-resistant cells (HT29/L-OHP2 and HT29/L-OHP20) were fixed with 70% ethanol, stained with propidium iodide, and subjected to flow cytometry.

about the roles of the up-regulated enzyme in chemoresistant cancer cells. We show evidence that the modulation of the isoprenoid metabolism by AKR1B10 is involved in proliferative capacity of the L-OHP-resistant HT29 cells (**Figures 5** and **6**). In the parental HT29 cells (**Figures 5A,B**), the treatments with low concentrations (1–2 μM) of farnesol and farnesal facilitate the cell growth, although their high doses (>5 μM) lower the viable cell numbers as reported by Joo and Jetten (2010). The facilitation of cell growth by the isoprenoids is further potentiated by the addition of 4-hydroxyacetophenone, an inhibitor of aldehyde dehydrogenase 3A2 that catalyzes oxidation of farnesal into farnesoic acid (Endo et al., 2011). In the analysis of DNA replication potential (**Figure 6**), the BrdU uptake of the parental cells is elevated by treating with farnesol or farnesal plus 4-hydroxyacetophenone. The two treatments also enhance the DNA replication potential of resistant HT29/L-OHP2 cells (which is originally high due to the overexpression of AKR1B10), but has no stimulatory effect on HT29/L-OHP20 cells with low AKR1B10 expression. In addition, activation of MEK1/2, also known as MAP kinase kinase, is observed in HT29/L-OHP2 cells treated with 1 μM farnesol or farnesal (data not shown). These results may indicate that the AKR1B10-mediated reduction of farnesal into farnesol not only lessens colon cancer cell damage induced by farnesoic acid, but also promotes the cell proliferation, probably due to protein farnesylation and activation of MAP kinase signaling as mentioned above (**Figure 1B**). Thus, AKR1B10 overexpressed in the resistant HT29/L-OHP2 cells participates in cell proliferation at least by modulating the isoprenoid metabolism.

ANTIOXIDANT CAPACITY

Treatment of cancer cells with some chemotherapeutic agents generates ROS, which modify biomolecules, nucleic acids, proteins and lipids, and consequently forms highly reactive and toxic carbonyl compounds, such as 4-hydroxy-2-nonenal and 4-oxo-2-nonenal. *In vitro* enzyme assay showed that AKR1B10 effectively reduces these lipid peroxidation-derived aldehydes into their corresponding alcohols (Liu et al., 2009a; Martin and Maser, 2009; Wang et al., 2009; Shen et al., 2011), of which metabolism of HNE by AKR1B10 is also verified in cell-based experiments (Zhong et al., 2009; Shen et al., 2011). Knockdown of AKR1B10 gene by small interference RNAs sensitizes colon cancer HCT-8 cells to acrolein and crotonaldehyde (Yan et al., 2007). While many studies support the detoxification of lipid peroxidation-derived carbonyl compounds as a role of AKR1B10 in tumor development, there



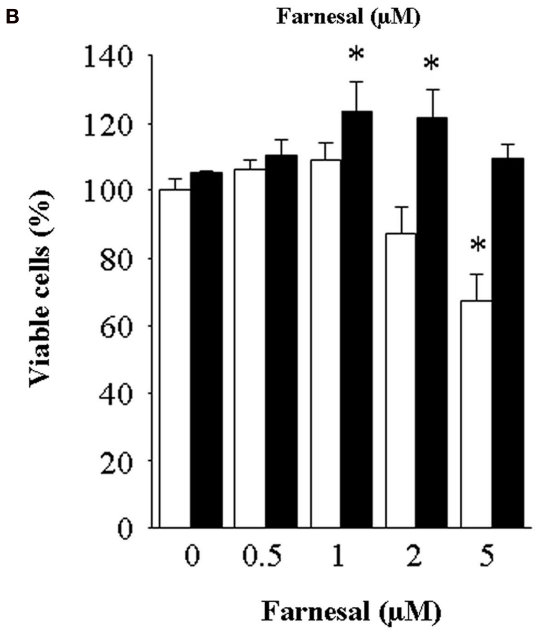


FIGURE 5 | Facilitation of proliferation potential of HT29 cells by treatment with farnesol (A) and farnesal (B). HT29 cells were pretreated for 2 h without (□) or with 25 μM 4-hydroxyacetophenone (■), and then treated for 48 h with the indicated concentrations of farnesol or farnesal. The viable cell number was evaluated as noted in **Figure 4**. Value is normalized to those in the control cells incubated without both isoprenoids and 4-hydroxyacetophenone, and expressed as the mean ± SD of three independent experiments. *Significant difference ($p < 0.05$) from the control cells.

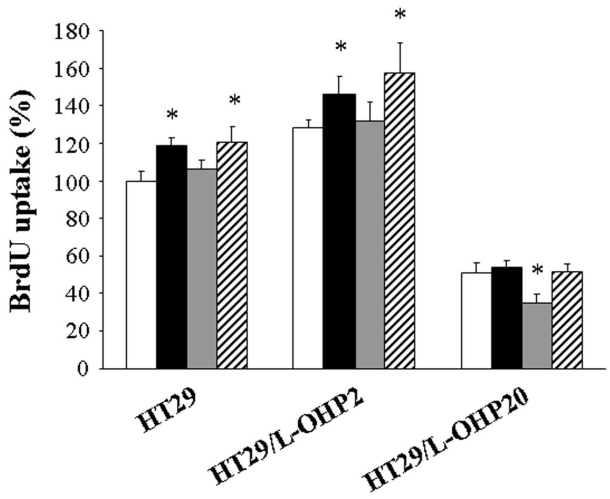


FIGURE 6 | Isoprenoids promote DNA replication in L-OHP-resistant HT29 cells. The parental HT29 cells and resistant HT29/L-OHP2 and HT29/L-OHP20 cells were treated for 48 h without (□) or with 1 μM farnesol (■) or 1 μM farnesal (▩), and the BrdU uptake was measured in the cells. The cells were also pretreated for 2 h with 25 μM 4-hydroxyacetophenone prior to treating with 1 μM farnesal (▨). Value is normalized to that in the control HT29 cells incubated without both isoprenoids and 4-hydroxyacetophenone, and expressed as the mean ± SD of three independent experiments. *Significant difference ($p < 0.05$) from the control cells.

is only one study concerning this role in chemoresistance. Mitomycin *c* produces ROS by its redox cycling, and the detoxification of lipid aldehydes by AKR1B10 is reported to be a major molecular basis for gaining of the mitomycin *c* resistance of HT29 cells (Matsunaga et al., 2011). It is possible that AKR1B10 exert this role in cancer cells resistant to other anticancer drugs, such as bleomycin (Khadir et al., 1999) and paclitaxel (Alexandre et al., 2007), that are known to produce ROS.

REVERSAL OF CHEMORESISTANCE BY AKR1B10 INHIBITORS

AKR1B10 is considered as a key factor responsible for carcinogenesis and chemoresistance as mentioned above. The enzyme also exhibits different substrate profiles from AKR1B1, despite their high structural homology. Therefore, development of potent inhibitors specific to AKR1B10 is prerequisite for remediation of the enzyme-related diseases. Recently, cyclopentenone prostaglandin A_1 has been reported to be an AKR1B10 inhibitor, which covalently binds to Cys299 near the active site of the enzyme (Díez-Dacal et al., 2011). Interestingly, the inhibitor seems to increase sensitivity of lung cancer A549 cells to doxorubicin. Another study showed that polyphenol butein potently inhibits DL-glyceraldehyde reductase activity of AKR1B10, with an IC_{50} value of 1.47 μM (Song et al., 2010). We previously reported steroid hormones, bile acids, and their metabolites as endogenous AKR inhibitors (Endo et al., 2009). More recently, by means of natural products-based comprehensive analyses and *in silico* screening approaches we have found curcumin derivatives (Matsunaga et al., 2009), a fluorone derivative (Zhao et al., 2010), chromene derivatives (Endo et al., 2010a), non-steroidal antiinflammatory agents (Endo et al., 2010b), and oleanolic acid (Takemura et al., 2011) to be potent and/or specific AKR1B10 inhibitors. Structures and IC_{50} values of representative AKR1B10 inhibitors are shown in **Figure 7**. Among the inhibitors, a chromene derivative, PHPC, is the most potent competitive inhibitor with an IC_{50} value of 6 nM, although the AKR1B10 selectivity versus AKR1B1 is approximately twofold.

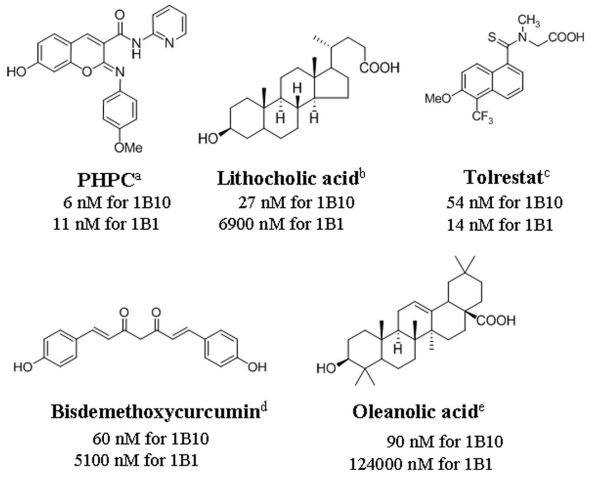


FIGURE 7 | Structures and IC_{50} values of representative AKR1B10 inhibitors. The IC_{50} values for AKR1B10 (1B10) and AKR1B1 (1B1) are taken from [a]Endo et al. (2010a), [b]Endo et al. (2009), [c]Endo et al. (2010b), [d]Matsunaga et al. (2009), [e]Takemura et al. (2011).

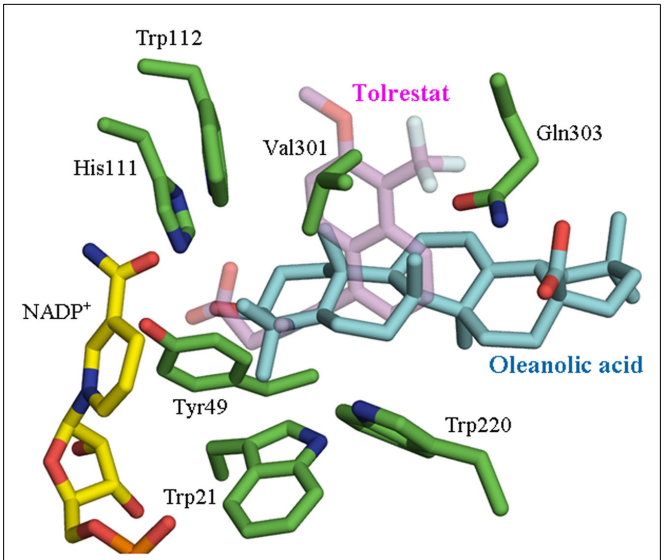


FIGURE 8 | Comparison of binding modes of oleanolic acid and tolrestat in the AKR1B10–NADP$^+$ complex. The oleanolic acid-docked model (Takemura et al., 2011) is superimposed on the crystal structure of tolrestat–NADP$^+$–AKR1B10 ternary complex (Gallego et al., 2007), and only tolrestat (pink) is shown with residues involving in the binding to oleanolic acid (sky-blue).

In contrast, oleanolic acid inhibits AKR1B10 with the highest selectivity ratio of 1370. The crystal structure of the enzyme-NADP$^+$–tolrestat ternary complex reveals that the inhibitor binds to the active site of the enzyme (Gallego et al., 2006). Like tolrestat (Endo et al., 2009), the above inhibitors are kinetically competitive inhibitors, suggesting that they also bind to the same site as that for tolrestat. **Figure 8** shows the oleanolic acid-docked model, on which tolrestat of the crystal structure was superimposed. The two inhibitors occupied the substrate-binding site of the enzyme, in which the 3β-hydroxy group of oleanolic acid and carboxyl group of tolrestat are in close proximity to catalytically important residues (Tyr49 and His111). There are differences in the orientation of the other parts of the two molecules, and two residues (Val301 and Gln303) are suggested to be key determinants of the inhibitory selectivity of oleanolic acid for AKR1B10 over AKR1B1 (Takemura et al., 2011). The cell-based approaches show that the two inhibitors (PHPC and oleanolic acid) remarkably reversed the mitomycin *c* resistance of HT29 cells (Matsunaga et al., 2011; Takemura et al., 2011). Thus, potent and selective AKR1B10 inhibitor would be useful for adjuvant medicine to subdue the development of cancer resistance to chemotherapy.

CONCLUDING REMARKS

It is commonly assumed that chemoresistance of cancer cells is induced with extraordinary enhancement of four capacities (1) to metabolize anticancer drugs into their harmless forms, (2) to protect from damage evoked by them, (3) to secrete them into extracellular space and (4) to grow in order to overcome their lethal effects through activation of growth factor signaling pathways, of which, AKR1B10 is involved in the three capacities (1, 2, and 4) as mentioned above. It is recently shown that the AKR1B10 activity is controlled by exchange of thiol–disulfide in Cys residues that are attacked by reactive radicals (Shen et al., 2010). In addition, analyses of the single-nucleotide polymorphism of AKR1B10 disclosed the presence of three kinds of mutations (P87S, M286T, and N313D), which impair reductase activity of the enzyme toward (*1S*)-1-indanol, albeit to a lesser degree (Bains et al., 2010). Thus, it is speculated that the alteration in expression and/or activity of AKR1B10 significantly influences on the manifestation of drug resistance and malignant progression. As mentioned above, there is limited direct evidence in the literature regarding expression profiles of the enzyme in cancers with chemoresistance. Additionally, definite roles of other four AKRs in chemoresistant cells have been not fully understood. Further studies are therefore needed to monitor the expression of AKRs in various kinds of cancers refractory to drugs.

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Biological role of aldo–keto reductases in retinoic acid biosynthesis and signaling

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Several aldo–keto reductase (AKR) enzymes from subfamilies 1B and 1C show retinaldehyde reductase activity, having low K_m and k_{cat} values. Only AKR1B10 and 1B12, with all-*trans*-retinaldehyde, and AKR1C3, with 9-*cis*-retinaldehyde, display high catalytic efficiency. Major structural determinants for retinaldehyde isomer specificity are located in the external loops (A and C for AKR1B10, and B for AKR1C3), as assessed by site-directed mutagenesis and molecular dynamics. Cellular models have shown that AKR1B and 1C enzymes are well suited to work *in vivo* as retinaldehyde reductases and to regulate retinoic acid (RA) biosynthesis at hormone pre-receptor level. An additional physiological role for the retinaldehyde reductase activity of these enzymes, consistent with their tissue localization, is their participation in β-carotene absorption. Retinaldehyde metabolism may be subjected to subcellular compartmentalization, based on enzyme localization. While retinaldehyde oxidation to RA takes place in the cytosol, reduction to retinol could take place in the cytosol by AKRs or in the membranes of endoplasmic reticulum by microsomal retinaldehyde reductases. Upregulation of some AKR1 enzymes in different cancer types may be linked to their induction by oxidative stress and to their participation in different signaling pathways related to cell proliferation. AKR1B10 and AKR1C3, through their retinaldehyde reductase activity, trigger a decrease in the RA biosynthesis flow, resulting in RA deprivation and consequently lower differentiation, with an increased cancer risk in target tissues. Rational design of selective AKR inhibitors could lead to development of novel drugs for cancer treatment as well as reduction of chemotherapeutic drug resistance.

Keywords: aldo–keto reductase, retinaldehyde, retinoic acid, retinol, cancer

INTRODUCTION

Members of the aldo–keto reductase (AKR) superfamily are NADP(H)-dependent cytosolic enzymes which fold into a typical $(\alpha/\beta)_8$-barrel. AKRs catalyze the reduction of a wide variety of carbonyl compounds (Jez et al., 1997; Barski et al., 2008). With regard to the biological function of human AKRs, they may be involved in the detoxification of electrophilic compounds, such as 4-hydroxy-*trans*-2-nonenal generated under oxidative stress conditions. AKRs are also able to reduce a variety of lipophilic substrates, such as ketosteroids, ketoprostaglandins, and retinoids, which are hormone precursors. Thus, another function may be related to pre-receptor hormone regulation of the amount of ligand available for some nuclear receptors and, therefore, to participation in transcriptional gene control. Lastly, they are considered as phase I drug-metabolizing enzymes and can either detoxify or activate xenobiotic compounds (i.e., polycyclic aromatic hydrocarbons; Penning and Drury, 2007).

With respect to the human AKRs, members of the AKR1B subfamily are of special interest. AKR1B1, the classical aldose reductase, is related to secondary diabetic complications, while AKR1B10 is induced in cancer cells. Although these two AKRs share 71% sequence identity, they show very different kinetic properties with some relevant substrates like retinaldehyde or glucose. Thus, AKR1B10 is a retinaldehyde reductase with a much higher k_{cat} value than that of AKR1B1, while glucose is reduced by AKR1B1 but not by AKR1B10. Recently, AKR1B10 has emerged as a tumor marker since it is overexpressed in different types of cancers, featuring hepatocellular carcinoma and lung cancer correlated with tobacco smoking (Ruiz et al., 2009; Wang et al., 2009; Díez-Dacal et al., 2011).

AKR1C1–AKR1C4, also known as human hydroxysteroid dehydrogenases, share 86% sequence identity, but the individual enzymes show different substrate specificity, inhibitor selectivity, and tissue expression pattern. AKR1C3 is one of the most interesting enzymes, with increasing evidence strongly supporting its involvement in cancer development (Jin and Penning, 2007; Penning and Byrns, 2009; Ruiz et al., 2011b).

The term "retinoid" refers to compounds derived from vitamin A (retinol), where their basic structure is divided into three domains: a cyclohexene ring or β-ionone, an aliphatic chain with

Abbreviations: ADH, alcohol dehydrogenase; AKR, aldo–keto reductase; ALDH, aldehyde dehydrogenase; BCO1, β-carotene 15,15′-monooxygenase 1; CRABP, cellular retinoic acid binding protein; CRBP-I, -II, cellular retinol binding protein type I, II; CYP26, cytochrome P450 family 26; LRAT, lecithin:retinol acyl transferase; MD, molecular dynamics; MDR, medium-chain dehydrogenase/reductase; PPARγ, peroxisome proliferator-activated receptor γ; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RAR, retinoic acid receptor; RBP, retinol binding protein; REH, retinyl ester hydrolase; ROS, reactive oxygen species; RXR, retinoid X receptor; SDR, short-chain dehydrogenase/reductase; STRA6, stimulated by retinoic acid gene 6; TTR, transthyretin.

four conjugated double bonds, and a polar group which may show different oxidation states. The different configuration of these double bonds leads to different stereoisomers, being the most frequent all-*trans*, 9-*cis*, 11-*cis*, and 13-*cis* (Blomhoff and Blomhoff, 2006). Retinol and its derivatives retinaldehyde and retinoic acid (RA) are essential for the growth and maintenance of many body tissues, such as skin, bone, and vasculature, as well as for the visual cycle (11-*cis*-retinaldehyde) and immune function. They also play a role in reproduction, embryonic growth, and development. In this sense, RA is a key molecule in the development of different vertebrate organs and tissues by promoting cell differentiation and apoptosis. RA has also a role in several pathological conditions, such as skin diseases, premature birth, and rheumatoid arthritis. Various human cancers have altered retinoid metabolism and low RA levels, which favor tumor progression (Theodosiou et al., 2010; Tang and Gudas, 2011).

Vitamin A incorporation in the body occurs through dietary intake of animal products, which are a source of retinyl esters, and from the consumption of fruits and vegetables, which are a source of carotenoids, mainly β-carotene (**Figure 1A**). In the first case, retinol is absorbed by the enterocyte cell after retinyl esters have been hydrolyzed in the small intestine. In the second case, β-carotene is absorbed directly by passive diffusion, and then in the enterocyte is split into two retinaldehyde molecules due

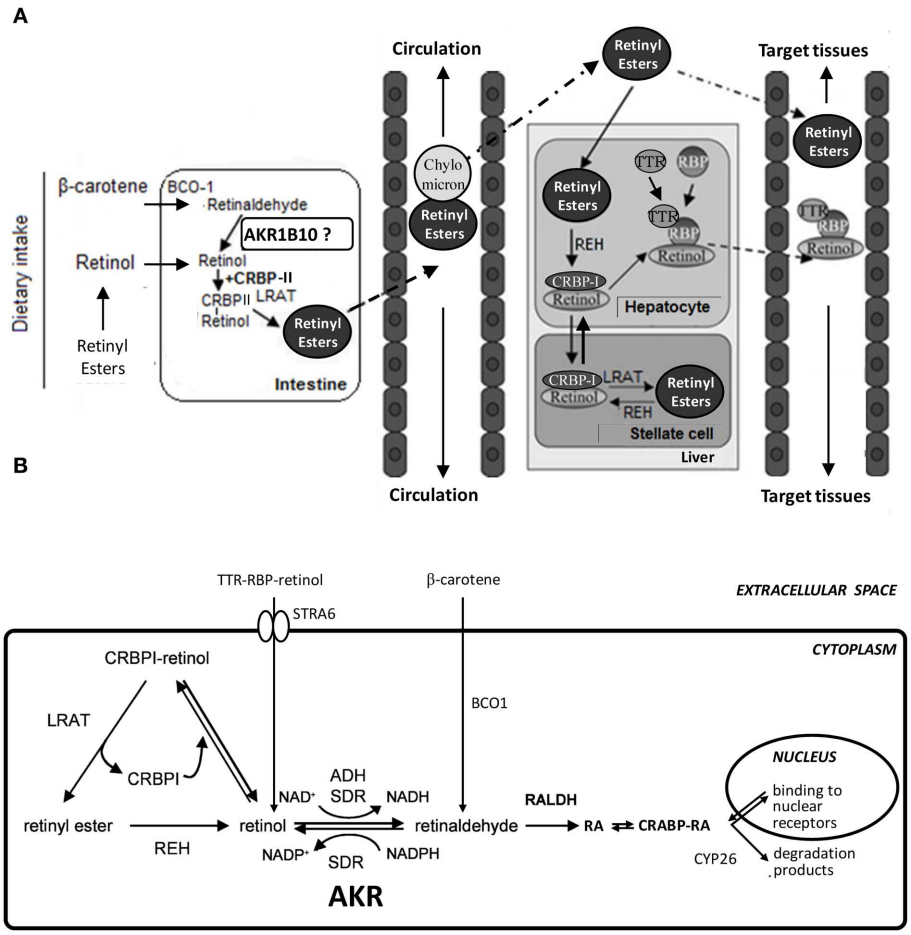

FIGURE 1 | Schematic diagram of retinoid absorption, transport, and metabolism. (A) Upon dietary intake and intestinal absorption, retinoids, and carotenoids are converted to retinol, which is bound to CRBP-II. Retinol is further processed to retinyl esters, incorporated into chylomicrons, and transported to the liver. In hepatocytes, retinyl esters are hydrolyzed to retinol which forms a complex with CRBP-I. Retinol can then be transferred to stellate cells for storage as retinyl esters. Alternatively, retinol may bind to RBP, and the retinol:RBP complex is bound to TTR and transferred to circulation for transport to target tissues. Binding to TTR reduces renal clearance. Adapted with permission from (Theodosiou et al., 2010). Copyright©2010, Birkhäuser Verlag, Basel. **(B)** In the target tissues, STRA6, a cell-surface receptor for RBP, facilitates retinol entry. Carotenoids may be also a major source of cellular retinoids. Levels of CRBP-I, LRAT, REH, and oxidoreductases (ADH, SDR, AKR, and RALDH) influence the retinoid flow either toward the storage pathway or the synthesis of RA, which is bound to CRABP. RA enters the nucleus, where it binds to nuclear receptors. RA is degraded and eliminated by CYP26 enzymes. Adapted with permission from (Parés et al., 2008). Copyright©2008, Birkhäuser Verlag, Basel. ADH, alcohol dehydrogenase; AKR, aldo–keto reductase; BCO1, β-carotene 15,15′-monooxygenase 1; CRABP, cellular retinoic acid binding protein; CRBP-I, -II, cellular retinol binding protein type I, -II; CYP26, cytochrome P450 family 26; LRAT, lecithin:retinol acetyltransferase; RA, retinoic acid; RALDH, retinaldehyde dehydrogenase; RBP, retinol binding protein; REH, retinyl ester hydrolase; SDR, short-chain dehydrogenase/reductase; STRA6, stimulated by retinoic acid gene 6; TTR, transthyretin.

to the activity of β-carotene 15,15′-monooxygenase 1 (BCO1). The liver is another tissue which contributes significantly to β-carotene cleavage. Later, retinaldehyde is reduced to retinol. In the cell, retinol has two metabolic alternatives: storage or oxidative metabolism. One form of storage is through binding to cellular retinol binding protein type I and type II (CRBP-I and II, respectively). CRBP-I shows wide tissue expression, while CRBP-II is expressed in the small intestine. In the enterocyte, retinol is attached to CRBP-II, and through the action of lecithin:retinol acyl transferase (LRAT) is esterified with fatty acids to yield long-chain esters of retinol. These are packaged into chylomicrons and are transported to the liver parenchymal cells where they are captured by specific receptors and transferred to stellate cells for storage, which constitutes 50–80% of body retinol in the form of retinyl esters. When required in the peripheral tissues, retinyl esters are hydrolyzed to retinol by retinol ester hydrolase (REH). The retinol generated is secreted and transported in the blood as a complex between retinol and the plasmatic retinol binding protein (RBP), which in turn is complexed with transthyretin (TTR) to reduce its glomerular filtration. Over 90% of retinol entering the cell is recycled to the plasma, and only a small part is esterified for storage, activated to RA or catabolized (Theodosiou et al., 2010; Harrison, 2012; Shirakami et al., 2012).

Biosynthesis of RA, the most potent biologically active metabolite of vitamin A, requires two oxidative steps (**Figure 1B**). Members of three oxidoreductase superfamilies have been implicated in the reversible oxidation of retinol to retinaldehyde, which is the pathway rate-limiting step (Napoli, 1999; Belyaeva et al., 2008). The participation of cytosolic alcohol dehydrogenases (ADH) from the medium-chain dehydrogenase/reductase (MDR) superfamily and of microsomal short-chain dehydrogenases/reductases (SDR) has been deeply studied. More recently, the AKR superfamily has been added as a novel group of cytosolic enzymes that could contribute to retinoid redox conversions. Based on the cofactor specificity, ADH (NAD-dependent) may work in the oxidative direction, AKRs (NADPH-dependent) in the reductive direction, while SDR show examples of both specificities.

The control of retinaldehyde levels is essential in the regulation of RA synthesis and, therefore, of its signaling role. Once retinaldehyde is synthesized, it has two alternative metabolic fates, its irreversible oxidation to RA by the action of aldehyde dehydrogenases (ALDH or RALDH) or its reduction back to retinol by retinaldehyde reductases. Synthesized RA binds to cellular RA binding protein (CRABP) and is transported to the cell nucleus where it binds to retinoid receptors. These can be divided into two subgroups: RA receptors (RAR), binding the isomers all-*trans* and 9-*cis*-RA, and retinoid X receptors (RXR), binding 9-*cis*-RA with high affinity, but not all-*trans*-RA. RAR/RXR heterodimers are bound to RA response elements (RARE) in DNA. Ligand binding induces a conformational change in the RAR/RXR heterodimers which promotes gene transcription. While RAR only forms heterodimers with RXR receptor (RAR–RXR), RXR is capable also to form heterodimers with thyroid hormone (RXR–TR), vitamin D (RXR–DR), and peroxisomal proliferator-activated (RXR–PPAR) receptors. It appears that the RXR activity is subordinated to the presence of ligand bound to RAR in the heterodimer (Pogenberg et al., 2005). There is a balance between synthesis and catabolism to control RA levels. RA catabolism to more oxidized metabolites such as 4-hydroxy-RA or 4-oxo-RA occurs primarily through enzymes from the CYP26 family (Parés et al., 2008; Theodosiou et al., 2010; Tang and Gudas, 2011; Kumar et al., 2012).

The first AKR enzyme reported to display activity with retinoids was chicken AKR or AKR1B12 (Crosas et al., 2001). Since then, a significant amount of data has been reported on AKRs and retinoids. In this review, this relevant topic will be discussed, as AKRs are being established as enzymes that participate in the precise fine tuning required for the first and regulatory step of RA biosynthesis. Regulation of this pathway by AKRs and its relationship with retinoid body uptake and cell proliferation will also be approached because of its pathological relevance.

CHARACTERIZATION OF AKRs AS RETINALDEHYDE REDUCTASES

ACTIVITY ASSAY METHODOLOGIES AND KINETIC RESULTS FOR AKR ENZYMES WITH RETINOIDS

In vitro kinetic studies on AKR enzymes with retinoids are fundamental to investigate isomer specificity, inhibitor selectivity, and structure–function relationships. Retinoids are highly unstable hydrophobic compounds displaying very low solubility in water-based solvents and being susceptible to photodegradation, double-bond isomerization, and oxidation reactions. Thus, they need to be handled under dim red light, and properly solubilized and stabilized. In order to overcome these difficulties, two different methodologies have been used to perform kinetic studies with retinoids: (1) the ADH enzymatic assay (or Tween 80 assay), and (2) the SDR enzymatic assay (or HPLC assay), both reviewed in Parés et al. (2008).

The ADH enzymatic assay (or Tween 80 assay)

This assay is characterized by the use of an aqueous buffer containing a low amount of the non-ionic detergent Tween 80 (polyoxyethylene (20) sorbitan monooleate) and the spectrophotometric measurement of the reaction at 25°C, following retinaldehyde absorbance at 400 nm, where retinol does not absorb. **Table 1** lists the k_{cat} values of the AKR1 enzymes obtained by using this method. K_m values are not included because Tween 80 behaves as an apparently competitive inhibitor and thus, at the concentration used in the assay, there is a 10- to 100-fold increase of the retinoid K_m values (Martras et al., 2004; Ruiz et al., 2009). Some of the values in **Table 1** may not be fully comparable since kinetic studies were performed in different laboratories using different protein sources and methodologies, under various experimental conditions. Thus, AKR1B12 results were obtained by using tissue-purified instead of recombinant protein. Constants for AKR1B13, 1B14, 1B17, and 1C15 were measured with 0.01% (v/v) Tween 80 instead of the widely used 0.02% concentration. Other laboratories used a high concentration of organic solvent (e.g., 8%, v/v, dimethylsulfoxide). Another added trouble with this methodology is its low reproducibility due to the high absorbance of retinaldehyde at 400 nm combined with the low activity rate shown by AKR1 enzymes, resulting in high and variable background which must be subtracted during activity assays. While this methodology may still serve to perform routine determination of enzyme activity, it is not useful for comparison of kinetic parameters with

Table 1 | Catalytic constants (k_{cat}, min^{-1}) of AKR1 enzymes with retinaldehyde isomers, obtained spectrophotometrically in the presence of Tween 80.

Enzyme	All-*trans*-retinaldehyde		9-*cis*-Retinaldehyde
1A2	N.A.[a]		N.A.[a]
1B1	0.37[b]		2.84
1B10	17.8[b]	39.0[c]	5.20
1B3	11.6[d]		N.D.
1B7	9.73[d]		N.D.
1B8	15.3[d]		N.D.
1B9	0.11		N.A.
1B12	17.0[e]		8.62[e]
1B13	N.A.[f]		N.D.
1B14	N.A.[g]		N.D.
1B17	0.58[h]		N.D.
1C7*	L.A.[i]		N.D.
1C15	1.2[j]		2.8[j]

*Activity was determined in 0.1 M sodium phosphate, pH 7.5, 0.02% Tween 80, 0.2 mM NADPH, at 25°C. ND, not determined; NA, no activity was detected or it was less than 0.5 nmol min^{-1} mg^{-1}. LA, low activity was detected, 4 nmol min^{-1} mg^{-1}. *Kinetics was performed in 0.1 M potassium phosphate buffer, pH 6.5, at 37°C, and products were analyzed by reverse phase HPLC equilibrated with 80% (v/v) acetonitrile in water. [a] Crosas et al. (2003), [b] Gallego et al. (2007b), [c] Quinn et al. (2008), [d] Joshi et al. (2010), [e] Crosas et al. (2001), [f] Endo et al. (2009), [g] Endo et al. (2010a), [h] Endo et al. (2010b), [i] Endo et al. (2001), [j] Endo et al. (2007).*

Table 2 | Kinetic constants of AKR1 enzymes with retinaldehyde isomers, obtained by the HPLC assay in the presence of BSA.

Enzyme	All-*trans*-retinaldehyde			9-*cis*-Retinaldehyde		
	K_m	k_{cat}	k_{cat}/K_m	K_m	k_{cat}	k_{cat}/K_m
1B1[a]	1.1	0.35	320	0.4	0.7	1500
1B3[b]	1.0	0.52	540		N.D.	
1B7[b]	0.5	0.02	42		N.D.	
1B8[b]	2.1	0.05	22		N.D.	
1B9[b]	2.0	0.27	140		N.D.	
1B10[a]	0.6	27	45000	0.7	0.9	1300
1B12	0.6	2.5	4100		N.D.	
1C1[c]		L.A.		0.48	0.18	370
1C2[c]		N.A.			N.A.	
1C3[c]	1.4	0.60	430	0.40	13	32,500
1C4[c]	0.31	0.24	790	0.80	0.40	500

Activities were determined in 90 mM KH_2PO_4, 40 mM KCl, pH 7.4, 0.5 mM NADPH, 37°C. NA, no activity; ND, not determined; LA, low activity was detected, 0.56 nmol min^{-1} mg^{-1}. Units: K_m (μM), k_{cat} (min^{-1}), k_{cat}/K_m (mM^{-1} min^{-1}). [a] Gallego et al. (2007b), [b] Ruiz et al. (2011a), [c] Ruiz et al. (2011b).

Table 3 | Kinetic constants of AKR1 enzymes with retinol isomers, obtained by the HPLC assay in the presence of BSA.

Enzyme	All-*trans*-retinol			9-*cis*-Retinol		
	K_m	k_{cat}	k_{cat}/K_m	K_m	k_{cat}	k_{cat}/K_m
1B10[a]	0.4	4.3	12300		N.A.	
1B12	0.5	1.4	2900		N.A.	
1C3[b]		N.A.		0.30	0.26	850

Activities were determined in 90 mM KH_2PO_4, 40 mM KCl, pH 7.4, 2.3 mM $NADP^+$, 37°C. NA, no activity. Units: K_m (μM), k_{cat} (min^{-1}), k_{cat}/K_m (mM^{-1} min^{-1}). [a] Gallego et al. (2007b), [b] Ruiz et al. (2011b).

other enzymes, such as SDR, where activity has been typically determined by using detergent-free buffer (Gough et al., 1998; Parés et al., 2008).

The SDR enzymatic assay (or HPLC assay)

This assay uses an aqueous buffer containing retinoid/bovine serum albumin at 1:1 molar ratio, reaction at 37°C, followed by retinoid extraction with hexane and analysis of the reaction products by HPLC. Determination of the reaction rate is based on the percentage of substrate conversion. Compared to the Tween 80 assay, the HPLC assay is far more reproducible, it does not alter the K_m values, requires a lower amount of substrate and is also suitable for cell culture experiments. Indeed, the comparative activity analysis side-by-side of ADH, SDR, and AKRs with retinoids revealed that all enzymes exhibited low and similar K_m values, 1 μM or lower, while they differed in their k_{cat} values. Therefore, when possible, it is highly recommended to choose this method instead of the Tween 80 assay.

Tables 2 and **3** show kinetic constants of AKR1 enzymes using the HPLC assay. As it can be observed, K_m values keep close to 1 μM or lower, which is in the physiological range of retinol concentration (Hollander and Muralidhara, 1977; Quick and Ong, 1990; Harrison, 2012). In contrast, k_{cat} values are clearly distinct between different enzymes (Gallego et al., 2006; Ruiz et al., 2011b). None of the rodent enzymes characterized displayed high k_{cat} values, leaving human AKR1B10 and chicken AKR1B12 as the sole potent all-*trans*-retinaldehyde reductases of the superfamily (Gallego et al., 2006, 2007b; Ruiz et al., 2011a). On the other hand, human AKR1C enzymes have been shown to use preferentially 9-*cis*-retinaldehyde, especially AKR1C3, which is unique in having a high k_{cat} value and a catalytic efficiency in the same order as that of AKR1B10 with all-*trans*-retinaldehyde (Ruiz et al., 2011b).

Some AKR1B enzymes are also active toward ring-oxidized retinoids, like all-*trans*-4-hydroxy, 4-oxo, and 3,4-didehydroretinaldehyde (Ruiz et al., 2009). Reminiscent of what is observed with ADH, k_{cat} values increase between 4- and 20-fold in AKRs with ring-oxidized retinoids in comparison with underivatized retinoids (Martras et al., 2004; Ruiz et al., 2009). These derivatives are involved in degradation pathways, but in some cases they are bioactive compounds which can bind to nuclear receptors. These results suggest that AKR1B enzymes may be potentially involved in these pathways *in vivo*.

EFFECT OF CRBP-I AND MICROSOMAL MEMBRANES ON RETINOID OXIDOREDUCTASE ACTIVITY

Cellular retinol binding protein type I is a cytosolic protein with a widespread tissue distribution. Since CRBP-I binds retinol with very high affinity (K_d for retinol ≈0.1 nM), retinol availability for

enzymes in a cellular environment is an important issue. Therefore, the activity of AKR1B10 with retinol, along with that of some other oxidoreductases from the MDR and SDR superfamilies, was checked in the presence of CRBP-I (Gallego et al., 2006) by using the HPLC assay. The presence of CRBP-I seriously hampered enzymatic activity with retinol. All the enzymes analyzed could use free retinol as a substrate but not retinol bound to CRBP-I. The small activity found with *holo*-CRBP-I was attributed to the dissociated free retinol which was in equilibrium with CRBP-I. This point was confirmed for microsomal RDH by other laboratories (Belyaeva et al., 2005, 2008; Farjo et al., 2011) and suggests that oxidoreductases may have limited access to retinol bound to CRBP-I.

In addition, evidence indicates that a fraction of cellular retinol is associated with membranes. In fact, retinol needs to be transferred to membranes of the endoplasmic reticulum in order to be esterified by LRAT, and most SDR retinol dehydrogenases are microsomal. Thus, the effect of added microsomal membranes on the retinol dehydrogenase activity of AKR1B10 and representative enzymes of two other oxidoreductase superfamilies was investigated. Enzyme activity was inhibited in a dose-dependent manner by microsomal membranes (Gallego et al., 2007a) and, in some cases, by adding phospholipid-based liposomes (Farjo et al., 2011). In conclusion, cytosolically located enzymes may not have access to retinol bound to CRBP-I nor to that absorbed into membranes, while microsomal enzymes could use the latter.

Retinaldehyde reductase activity of AKR1B1 and 1B10, side-by-side with that of cytosolic ADH and microsomal SDR enzymes, was also analyzed in the presence of CRBP-I (Gallego et al., 2006). Although the enzymes examined only could use free retinaldehyde, they could still function in the presence of CRBP-I because of a higher dissociation constant (K_d for retinaldehyde ≈50 nM), which results in a significant fraction of free retinaldehyde found in solution (Parés et al., 2008).

STRUCTURAL DETERMINANTS OF RETINALDEHYDE REDUCTASE ACTIVITY IN AKRs

AKR1B1 is likely one of the best characterized proteins at structural level, with a large number of high quality structures solved and some at unprecedented ultrahigh resolution (0.66 Å; Howard et al., 2004). Many crystallographic structures of AKR1 ternary complexes have been solved using enzyme inhibitors as ligands, mostly because of their pharmacological relevance (Lovering et al., 2004; El-Kabbani and Podjarny, 2007; Barski et al., 2008). In contrast, only one crystal structure of an AKR1B enzyme complex with a substrate or analog has been obtained (AKR1B1–NADP-glucose-6-phosphate, PDB 2ACQ) and only few for some AKR1C enzymes. In this latter case, the studies were based on the use of substrates showing low k_{cat} values, which helped stabilizing the ternary complex (i.e., AKR1C2–NADP-testosterone, PDB 1J96; AKR1C3–NADP-prostaglandin D_2, PDB 1RY0). As for retinoid molecules bound to AKR enzymes, structural data are lacking and only have been inferred from docking and molecular dynamics (MD) simulations (Gallego et al., 2007b; Ruiz et al., 2009, 2011a,b). From these crystallographic and modeling studies, the main interacting residues in the cofactor- and substrate-binding sites could be identified. In the active site, the vast majority of AKRs have a catalytic tetrad featuring Asp44, Tyr49, Lys78, and His111 (AKR1B10 numbering). The most variable area in the substrate-binding site between different enzymes is found in loops A (residues 112–136), B (residues 212–226), and C (residues 297–307). Substrate specificity and inhibitor selectivity is mostly determined by the interaction with residues located in these three highly variable loops.

Members of subfamily AKR1A (aldehyde reductase) did not show activity with retinoids. A larger loop C, with an insertion of nine amino acid residues, not present in AKR1B and AKR1C enzymes, could be the determinant for the absence of activity of pig aldehyde reductase (AKR1A2) with retinoids, as it restricts the access of bulky substrates and inhibitors to the cavity (Barski et al., 1996; Crosas et al., 2003).

Regarding the subfamily 1B, as mentioned above, several AKR1B1 inhibitor complexes have been obtained. In contrast, for AKR1B10, only one three-dimensional structure, the ternary complex AKR1B10–$NADP^+$-tolrestat has been solved (PDB 1ZUA; Gallego et al., 2007b). The comparison between AKR1B1 and AKR1B10 structures allowed explaining the difference of 100 times in catalytic efficiency with all-*trans*-retinaldehyde. MD simulations showed that all-*trans*-retinaldehyde is a bulky molecule which, in contrast to smaller molecules like tolrestat, interacted through its cyclohexene ring with the outer region of the substrate-binding pocket, especially with loops A and C. The most remarkable amino acid substitutions in residues participating in retinoid binding between AKR1B1 and AKR1B10 were observed at positions 125 (Leu to Lys) and 304 (Cys to Ser).

Site-directed mutagenesis exchanging the AKR1B10 residues for those of AKR1B1 (K125L and S304C) was performed and the resulting single and double mutant enzymes were kinetically characterized (**Table 4**). With all-*trans*-retinaldehyde, the k_{cat} value of both single mutants decreased more than 10 fold compared to the wild-type enzyme, while the double mutant showed a similar value to that of the low-retinoid activity AKR1B1. The kinetics with DL-glyceraldehyde was not affected by the substitutions likely because these residues are located far from where small substrates bind. Thus the k_{cat} value for DL-glyceraldehyde was similar for all the enzymes listed in **Table 4**, suggesting a common rate-limiting step, likely cofactor dissociation. In contrast, large differences in the k_{cat} value with all-*trans*-retinaldehyde suggest that the rate-limiting

Table 4 | Catalytic constants (k_{cat}, min^{-1}) of AKR1B10, AKR1B10 K125L, and AKR1B10 S304C mutants, and AKR1B1.

Enzyme	DL-Glyceraldehyde	All-*trans*-retinaldehyde
AKR1B10	35	27
K125L	35.6	2.0
S304C	29	2.0
K125L/S304C	28	0.12
AKR1B1	31	0.35

Activities were determined in 0.1 M sodium phosphate, pH 7.5, 0.2 mM NADPH, 25°C, with DL-glyceraldehyde, and in 90 mM KH_2PO_4, 40 mM KCl, pH 7.4, 0.5 mM NADPH, 37°C, with all-trans-retinaldehyde, using the HPLC assay. Data from Ruiz et al. (2011a).

step for this substrate differs between AKR1B1 and AKRB10. It is conceivable that either the chemical step or product release is slower when retinaldehyde is the substrate in AKR1B enzymes, the exception being AKR1B10, which has a similar k_{cat} value for all-*trans*-retinaldehyde and DL-glyceraldehyde. MD simulation and kinetics with ring-oxidized retinoids supported this notion, although transient kinetic experiments would be required to confirm it unequivocally (Gallego et al., 2007b; Ruiz et al., 2009, 2011a).

Molecular dynamics simulations showed that binding of all-*trans*-retinaldehyde to AKR1B10 required Lys125 to swivel toward the solvent, something not required in the other models tested (**Figure 2**; Gallego et al., 2007b). One interesting finding is that in order to achieve a higher enzyme activity with retinoids, AKR1B enzymes require a hydrophilic group to be present either in the substrate cyclohexene ring or in enzyme residue 125, facilitating proper orientation of substrate for catalysis or product release. When exchanging Ser304 by Cys in AKR1B10, some hydrogen bonds and hydrophobic interactions between loops A and C of AKR1B1 are predicted to be broken. Somehow, structural variations in the K125L/S304C double mutant may have induced protein loop rearrangements and product–protein interaction changes (Ruiz et al., 2011a), which may explain the large decrease in its k_{cat} value, similar to that of the low-retinoid activity AKR1B1 (**Table 4**). Thus, Lys125 and Ser304, the latter being unique to 1B10 and chicken 1B12 (also a highly active retinaldehyde reductase), are major structural determinants for all-*trans*-retinaldehyde specificity of 1B10, as assessed by site-directed mutagenesis and molecular dynamics. In summary, retinoid specificity in the 1B subfamily seems to be governed by hydrophilic contacts in the outer part of the substrate-binding site and by loop–loop interactions.

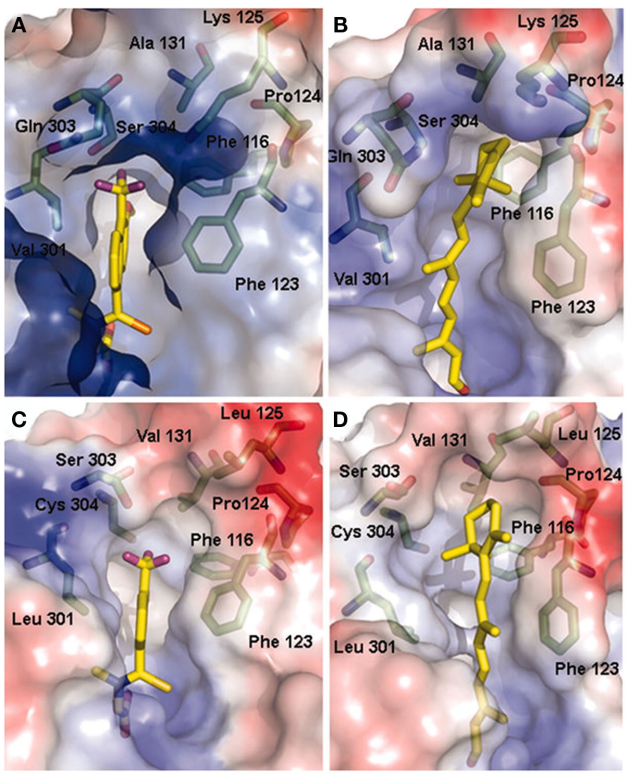

FIGURE 2 | Models of all-*trans*-retinaldehyde docked into the AKR1B10 and AKR1B1 structures. (A) Tolrestat-binding pocket in the AKR1B10–$NADP^+$-tolrestat crystal. **(B)** All-*trans*-retinaldehyde binding pocket of AKR1B10 predicted by our model. **(C)** Tolrestat-binding pocket in the AKR1B1–$NADP^+$-tolrestat crystal (PDB entry 2FZD). **(D)** All-*trans*-retinaldehyde binding pocket of AKR1B1 predicted by docking and MD. The molecular surface is colored according to the local electrostatic potential as calculated with the program PYMOL (www.pymol.org). Residues around the substrate define a highly hydrophobic and well adjusted pocket, protecting the retinaldehyde molecule from the polar solvent. Reproduced with permission from Gallego et al. (2007b). Copyright©2007, National Academy of Sciences, USA.

As described above, human 1C subfamily members, especially AKR1C3, are predominantly 9-*cis*-retinaldehyde reductases. AKR1C enzymes have some amino acid insertions at their N-terminal region and also a shorter loop B in the part interacting with the cofactor. This smaller loop makes the chemical step and product release to be more rate-limiting than cofactor dissociation in comparison to 1A and 1B enzymes (Jin and Penning, 2006; Barski et al., 2008). Binding of retinaldehyde isomers to AKR1C1, a low activity enzyme, and AKR1C3, a high activity enzyme, was compared and the computer models obtained were very similar in their overall structure, but with some subtle changes. Trp227, located in loop B and conserved in the two proteins, adopted different conformations in the complexes, resulting in different loop arrangements. Structural analysis suggested that the variation in the conformation could be due to residue 226, Arg in AKR1C3, and Pro in AKR1C1, resulting in a more rigid and tighter loop B. The importance of this residue was partially supported by a moderate drop in the catalytic efficiency of AKR1C3 R226P and R226Q mutants. Additional structural features might contribute to the k_{cat} difference between the two enzymes. Probably, the wider and more hydrophilic site of AKR1C3 could facilitate binding of 9-*cis*-retinaldehyde. The lower activity of AKR1C3 with all-*trans*-retinaldehyde could also be explained by a distinct loop B conformation in the complex with this isomer, whose different geometry resulted in a positioning similar to that of the AKR1C1:9-*cis*-retinaldehyde complex (Ruiz et al., 2011b).

BIOLOGICAL ROLE OF AKRs AS RETINALDEHYDE REDUCTASES

RETINALDEHYDE REDUCTASE ACTIVITY OF AKRs IN CELLULAR MODELS

Since AKR enzymes had been characterized *in vitro* as retinaldehyde reductases, their activity was also tested in different cellular models, namely, primary cell cultures as well as tumor cell lines. In order to identify endogenous or transfected AKRs as the origin of retinaldehyde reductase activity, two different experimental approaches were used, i.e., enzyme overexpression and/or the use of enzyme inhibitors.

Primary cultures of human aortic smooth muscle cells, when stimulated to proliferate, overexpressed AKR1B1 and converted 35% of added retinaldehyde to retinol. This conversion decreased by 40% when cells were incubated in the presence of tolrestat, an AKR1B1 inhibitor. Therefore, AKR1B1, which typically shows low

in vitro enzyme activity, acted as a retinaldehyde reductase in a cellular environment, which points out to a significant role *in vivo* (Gallego et al., 2006).

Monkey kidney COS-1 cells, when transiently expressing AKR1B10, doubled their capacity for all-*trans*-retinaldehyde reduction (Gallego et al., 2007b). Overexpression of AKR1B10 in human airway epithelial 16HBE cells treated with retinaldehyde also increased retinol production (Wang et al., 2010). Lastly, human lung adenocarcinoma A549 cells showed a retinaldehyde reducing activity of 9.6 ± 0.8 nmol min^{-1} mg^{-1}, which was inhibited by 50% upon treatment with ponalrestat (an AKR1B1 and AKR1B10 inhibitor; Quinn et al., 2008).

Breast adenocarcinoma MCF-7 cells were used as a model to study retinaldehyde reductase activity of AKR1C enzymes. These cells exhibit very low retinol oxidation activity, down-regulated retinol esterification and low retinaldehyde oxidation. Retinaldehyde reduction was found to be very high and approximately 30% of this activity was due to AKR1C enzymes, presumably to AKR1C3, as shown by flufenamic acid inhibition (an AKR1C1 and AKR1C3 inhibitor). The reductive metabolism of 9-*cis*-retinaldehyde was less significant, although AKR1C3 and AKR1C1 were responsible for approximately 90% of this activity (Ruiz et al., 2011b).

As for the oxidizing activity, transfection in COS-1 cells with AKR1B10 did not increase conversion of externally added retinol to retinaldehyde (Gallego et al., 2007b) and AKR1C3 inhibition had no effect on the retinol conversion in MCF-7 cells (Ruiz et al., 2011b). Thus, both AKR1B and AKR1C enzymes could not function as retinol dehydrogenases in cell culture. These results were expected since, in metabolically active cells, NADP is mostly found in its reduced state and therefore reductive metabolism by NADP-dependent enzymes is favored over oxidative reactions (Barski et al., 2008). A similar finding had been previously described in COS-1 cells, where transient transfection of AKR1C enzymes indicated that they function as ketosteroid reductases (Penning et al., 2004).

Overall, these studies using different cellular models confirm that the retinaldehyde reductase activity of human AKRs may have an *in vivo* role in the RA biosynthetic pathway.

EFFECT OF AKR ACTIVITY ON RA SIGNALING THROUGH PRE-RECEPTOR REGULATION

Having demonstrated that AKRs are able to decrease *in vitro* and cellular retinaldehyde levels, we explored whether their retinaldehyde reductase activity might also deplete RA levels thus affecting RA signaling. For this purpose, HeLa cells were transiently cotransfected with an AKR expression plasmid and a RARE reporter plasmid, and treated with either all-*trans* or 9-*cis*-retinol. Overexpression of each of AKR1B1, 1B10, 1C3, and 1C4 decreased both all-*trans*- and 9-*cis*-RA-dependent *trans*-activation, meaning a lower amount of RA being produced due to their retinaldehyde reductase activity (Ruiz et al., 2009, 2011b). This result implies that AKR1B and 1C enzymes are part of a fine tuning regulating system of RA biosynthesis, carried out by proteins from different superfamilies, such as AKR, MDR, and SDR, and described earlier in this review. Overall this indicates that AKR enzymes may contribute to pre-receptor down-regulation of RAR and RXR transcriptional activity, which could lead to cell proliferation and increased cancer risk in target tissues.

It is well known that these enzymes and other members of the human AKR1A, 1B, and 1D subfamilies are able to use as substrates lipophilic molecules other than retinoids, including steroids, prostaglandins, and polycyclic aromatic hydrocarbons. Interestingly, these compounds or their derivatives act as ligands of a wide variety of nuclear hormone receptors (Penning and Drury, 2007), which suggests a biological function in the regulation at pre-receptor level. Consequently, the retinaldehyde reductase activity of AKR1B and 1C enzymes can now be added to this complex regulatory interplay.

ROLE OF AKR1C3 IN THE CONTROL OF 9-*cis*-RA LEVELS

The 9-*cis* isomer of RA binds to both RAR and RXR with high affinity *in vitro*, and has diverse pharmacological actions which are distinct from those of all-*trans*-RA. Intensive analytical research in quantifying RA isomers had not detected 9-*cis*-RA in serum and in a variety of tissues, until the recent identification of this isomer in pancreas, where it plays a role in regulation of glucose-stimulated insulin secretion (Kane et al., 2010; Kane, 2012). About 80% of pancreatic 9-*cis*-RA is concentrated in β-cells, which represent less than 5% of the total pancreatic cell population. This indicates a very discrete cellular localization of this isomer. It is possible that 9-*cis*-RA may only be present in a local cell population in other tissues, and thus dilution of the isomer in the homogenate of the whole tissue may preclude its detection. A source of 9-*cis*-RA could be 9-*cis*-retinol, which has been detected in pancreas and other tissues (Kane, 2012), while enzymes oxidizing 9-*cis*-retinol and 9-*cis*-retinaldehyde are widespread (**Table 5**). In addition, 9-*cis* carotenoids found in the diet can produce 9-*cis*-retinaldehyde by the action of BCO1 (Kane, 2012). Levels of 9-*cis*-retinaldehyde would be controlled by the action of oxidoreductases. In general retinaldehyde oxidoreductases are more specific for the all-*trans* than for the 9-*cis* isomer (**Table 5**), except for several AKR enzymes, especially AKR1C3. The robust AKR1C3 activity with the 9-*cis* form is comparable or higher than that of the members of other enzyme superfamilies, supporting a role in the control of 9-*cis*-RA signaling. Specificity for the 9-*cis* over the all-*trans* isomer has also been observed in other enzymes, such as RDH5 (Mertz et al., 1997) and ALDH8A1 (Lin and Napoli, 2000).

ROLE OF CYTOSOLIC AKRs IN SUBCELLULAR RETINALDEHYDE METABOLISM

As discussed above, *in vitro* and cellular studies indicate that AKRs could be involved in the reduction of retinaldehyde to retinol. Furthermore, this activity could modulate RA synthesis, confirming that the control of retinaldehyde levels is essential in the regulation of RA function.

Available evidence supports cellular compartmentalization of retinoid metabolism. The enzymes involved in RA synthesis are localized in different subcellular compartments. In addition, the low solubility of retinol and retinaldehyde in water also influences their distribution in the cell. In the cytoplasm, retinol is tightly bound to CRBP-I (Napoli, 1999). Retinol is also found in free form incorporated into endoplasmic reticulum membranes, which is supported by the observation that CRBP-I can transfer retinol

Table 5 | Properties of human retinaldehyde oxidoreductases with reported kinetic constants.

Enzyme	Substrate				Reaction (cofactor)	Subcellular localization	Tissue distribution	Reference
	All-*trans*-Ral		9-*cis*-Ral					
	k_{cat}/K_m	V_{max}/K_m	k_{cat}/K_m	V_{max}/K_m				
AKR1B1	320	8.3	1500	45	Reduction (NADPH)	Cytosolic	Widespread	Gallego et al. (2007b), Grimshaw and Mathur (1989)
AKR1B10	45000	1200	1300	33			Small intestine, adrenal gland, colon ≫ liver, thymus	Gallego et al. (2007b), Hyndman and Flynn (1998), Cao et al. (1998)
AKR1C1	L.A.		370	10			Lung, liver ≫ testis, mammary gland	Ruiz et al. (2011b), Penning et al. (2000)
AKR1C3	430	11	32,500	850			Mammary gland, prostate ≫ liver, lung	Ruiz et al. (2011b), Penning et al. (2000)
AKR1C4	790	20	500	13			Liver	Ruiz et al. (2011b), Penning et al. (2000)
RDH11	N.D.	4200	N.D.	8.4*	Reduction (NADPH)	Microsomal	Widespread	Belyaeva et al. (2003), Kedishvili et al. (2002)
RDH12	900000	25000	100000	2800			Retina ≫> kidney > pancreas ≫ other	Belyaeva et al. (2005)
RDH14	N.D.	340*	N.D.				Widespread	Belyaeva and Kedishvili (2002)
ALDH1A1	N.D.	3700, 4.2**	N.D.	5.6**	Oxidation (NAD^+)	Cytosolic	Widespread	Yoshida et al. (1992), Xi and Yang (2008), King and Holmes (1998), Gagnon et al. (2002)
ALDH1A2	N.D.	6.5†	N.D.	1.2†			Testis, ovary > pancreas, placenta ≫ lung, intestine, liver	Xi and Yang (2008), Gagnon et al. (2002)
ALDH1A3	1170000	5000	N.D.				Widespread	Xi and Yang (2008), Graham et al. (2006)
ALDH8A1	L.A.#		N.D.	0.23#			Kidney and liver	Lin and Napoli (2000)

*All activities were measured using purified enzymes unless indicated otherwise. k_{cat}/K_m, $min^{-1}\,mM^{-1}$; V_{max}/K_m, $nmol\,min^{-1}\,mg^{-1}\,\mu M^{-1}$; k_{cat} values of AKRs were calculated using Mr = 38500; Values with purified SDR enzymes were taken from Parés et al. (2008); ND, not determined; LA, low activity; Ral, retinaldehyde; *Kinetic constants using microsomal fractions of transiently transfected cells; **Kinetics were performed with the presence of Tween 80 in the activity buffer; † Kinetic constants of mouse enzyme; # Kinetic constants using whole cell lysates of transiently transfected cells.*

to phospholipid membranes (Herr et al., 1999). LRAT and REH are both membrane-bound enzymes and LRAT-enriched microsomal fraction uses efficiently retinol bound to membranes or to CRBP-I (Ghyselinck et al., 1999; Gallego et al., 2006). As we have previously seen, the human enzymes involved in the redox transformations of retinol and retinaldehyde are not active with the CRBP-I-retinol complex, but only with free retinol (Gallego et al., 2006; Farjo et al., 2011). In fact, CRBP-I is not needed for retinol oxidation since mice lacking CRBP-I did not exhibit decreased RA synthesis but instead they had greatly reduced their stores of liver retinyl esters (Ghyselinck et al., 1999). Interestingly, double transgenic mice lacking CRBP-I and ADH recovered normal levels of retinyl esters, meaning that CRBP-I might protect free retinol from being oxidized to retinaldehyde and converted to RA. Interestingly, mouse loss-of-function models indicate that the highly active cytosolic ADH show a minor role in RA synthesis during embryonic development, while the less active microsomal RDH10 (an SDR member, also inactive with CRBP-I-bound retinol) is essential (Farjo et al., 2011; Kumar et al., 2012). This suggests that RDH10, but not ADH, has access to free retinol absorbed into the membrane, and that the physiological oxidation of retinol occurs predominantly in a membrane-bound cellular compartment (Farjo et al., 2011).

As opposed to retinol, retinaldehyde metabolism has distinct localization features. As we have discussed previously, CRBP-I binds less tightly retinaldehyde (Gallego et al., 2006; Kane et al., 2011), which can be more easily released to its free form than retinol in the cytoplasm. A physiological implication of this fact is that the presence of CRBP-I appears to favor retinaldehyde metabolism over that of retinol oxidation in the cytosol. Oxidation to RA is a cytosolic step since the four ALDH involved (1A1, 1A2, 1A3, and 8A1) are soluble proteins. Interestingly, although additional ALDH are localized in other subcellular compartments (e.g., endoplasmic reticulum, mitochondria, or peroxisomes), they are

not active with retinaldehyde. In contrast, retinaldehyde reduction could be catalyzed either by microsomal SDR or by the cytosolic AKRs. The contribution of each enzyme, and the direction (oxidation or reduction) of the reaction, will depend on its amount in a specific tissue in a precise moment during fetal development or adult life, and on its kinetic properties. **Table 5** lists the kinetic and localization properties of major enzymes of retinaldehyde redox transformations. Although comparison of kinetic constants should be done with caution because of different methodologies used, it is apparent that SDR and ALDH are the most efficient enzymes. Using knockout animals, it has been well demonstrated that the three most active ALDH involved are essential for normal embryonic development of mice (Farjo et al., 2011; Kumar et al., 2012). It is also clear that AKR1B10 and AKR1C3 exhibit remarkable efficiencies, comparable to those of enzymes from the other superfamilies. Thus, in tissues with increased expression, either normal tissues or in the pathological conditions discussed below, participation of AKR1B10 and AKR1C3 in the control of retinaldehyde levels could be relevant, which would directly affect the RA signaling pathway.

In summary, collected data situates the first step of RA biosynthesis (retinol to retinaldehyde) mainly in the membrane of endoplasmic reticulum, while the second step (retinaldehyde oxidation) is cytosolic. Finally, retinaldehyde reduction, which regulates the flow of RA synthesis, can happen in either subcellular compartment, with the likely contribution of cytosolic AKRs.

PUTATIVE FUNCTION OF AKRs IN THE METABOLISM OF DIETARY PROVITAMIN A CAROTENOIDS

Dietary carotenoids are a major source of vitamin A metabolites. In humans, about one half of absorbed β-carotene is cleaved in the intestinal mucosa by BCO1 to yield two molecules of retinaldehyde. This is then reduced to retinol by poorly characterized reductases, esterified, and the resulting retinyl ester incorporated into chylomicrons. Intact carotenoids can be directly incorporated into chylomicrons and delivered to the liver, and in association with lipoproteins can be also taken up by extrahepatic tissues (Harrison, 2012; Lobo et al., 2012). BCO1 is abundant in intestine and liver, but it is also expressed in a variety of tissues in the embryo and adult, suggesting that local specific conversion of β-carotene may contribute to retinoid metabolism in peripheral tissues (Lobo et al., 2012). AKR1B10 and other AKRs are also present in intestine and in several tissues (**Table 5**), and could have a role in the reduction of retinaldehyde produced by the BCO1 action. Furthermore, the cytosolic localization of the BCO1 catalyzed reaction may favor the participation of either AKRs or ALDH in the metabolic fate of retinaldehyde.

ROLE OF RETINALDEHYDE REDUCTASE ACTIVITY OF AKRs IN PROLIFERATION AND TUMORIGENESIS

Upregulation of some AKR1 enzymes in different types of cancer has been widely reported (reviewed in Ruiz et al., 2009, 2011b). AKR1B10 was initially identified as a protein expressed in hepatocellular carcinoma and its mRNA was also found elevated in a number of cancer cell lines, especially in non-small cell lung cancer and adenocarcinoma (Zeindl-Eberhart et al., 2004; Fukumoto et al., 2005). AKR1B1 has been shown to be involved in colon carcinomas (Tammali et al., 2006). AKR1C3 was overexpressed in a wide variety of tumors, including breast and prostate cancer, either in an hormone-dependent or independent manner (Penning and Byrns, 2009) and leukemia (Desmond et al., 2003).

Generally, the induction of AKR1 enzymes could be explained as a part of the cellular defense response against oxidative stress (Jin and Penning, 2007). Tobacco components and other xenobiotics generate reactive oxygen species which induce AKR1 enzymes in their role as phase I drug-metabolizing enzymes (**Figure 3**). Indeed, *AKR1B1*, *AKR1B10*, and *AKR1C1–AKR1C3* belong to the battery of genes regulated by antioxidant response elements via the Nrf2–Keap-1 complex in humans (Jin and Penning, 2007; Penning and Lerman, 2008; MacLeod et al., 2009; Ebert et al., 2011). AKR1 enzymes, in some cases, contribute to chemotherapeutic drug resistance, which could be rationalized by a direct action through enzymatic activity in the case of carbonyl-bearing compounds, i.e., daunorubicin (Novotna et al., 2008; Verma et al., 2008; Zhong et al., 2011), or indirectly, by reducing oxidative stress generated by these agents (Deng et al., 2004). Consequently, AKR1 enzyme inhibitors could be useful since they would be able to enhance the therapeutic efficacy of anticancer drugs by preventing chemoresistance (Jin and Penning, 2007; Martin and Maser, 2009; Wang et al., 2009; Shen et al., 2011).

A putative mechanism by which the activity of AKR1 enzymes could promote tumor growth is the conversion of retinaldehyde to retinol which would provoke RA deprivation and blockage of its differentiating effect, promoting cell proliferation, and fostering tumorigenesis (**Figure 3**). According to the model for retinoid pre-receptor metabolism and regulation in target tissues, CRBP-I acts favoring retinol storage into membranes and because of its high affinity for retinol and low one for retinaldehyde restricts the availability of the first and allows retinaldehyde reductases to display their activity (Parés et al., 2008). Overexpression of AKR1 enzymes found in some cancers would favor retinaldehyde reduction (Penning, 2005). In fact, lung adenocarcinoma cell line A549 possesses a robust retinaldehyde reductase activity, due mostly to AKR1B10 (and to AKR1B1 to a lesser extent; Quinn et al., 2008).

Regarding AKR1B10, due to its high catalytic efficiency with all-*trans*-retinaldehyde, the importance of the RA-related effect on tumorigenesis is a strong possibility. RA is a crucial factor in airway epithelial differentiation and its local deficiency could promote carcinogenesis of the airway epithelium. Upregulation of AKR1B10 may be an early event in carcinogenesis, as its expression is elevated in squamous metaplasia and precancerous lesions of non-small cell lung carcinoma. This is why it has been suggested as an early detection marker and treatment target for non-small cell lung carcinoma (Fukumoto et al., 2005). Also, AKR1B10 is a smoking-responsive gene, and its upregulation is not limited to squamous metaplasia and cancer tissues but occurs in the airway epithelium in response to smoking (even healthy smokers show induction of AKR1B10 expression). AKR1B10 high activity would also explain the failure of β-carotene supplementation to reduce lung cancer risk, because AKR1B10 would prevent RA generation (Penning, 2005; Quinn et al., 2008).

AKR1C3 induction is related to hormone-dependent breast and prostate cancer and now with a putative relevant role in cancer

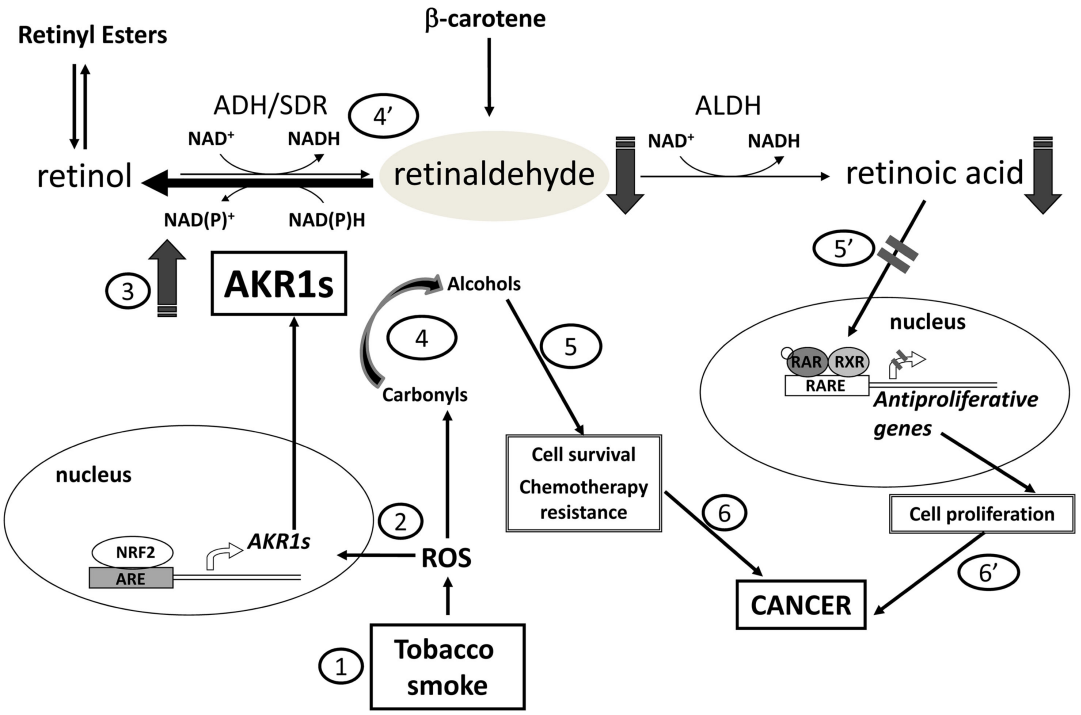

FIGURE 3 | Relationship of AKR1 enzymes with carcinogenesis at different levels. Firstly, reactive oxygen species (ROS), such as these generated by tobacco smoke, trigger the expression of some AKR1 enzymes (steps 1, 2, 3). Subsequently, activity of AKR1 enzymes with retinaldehyde provokes retinoic acid (RA) deprivation blocking its differentiating effect (steps 4′ and 5′), and favoring cell proliferation. Besides, AKR1 enzymes participate in the detoxification of lipid peroxidation aldehydes and can also metabolize various antitumoral agents bearing a carbonyl group (step 4). Through these activities, the enzymes promote cell survival and chemotherapeutic drug resistance (step 5). Overall, induction of AKR1 enzymes foster tumorigenesis (steps 6 and 6′).

related to RA signaling. Recently, cell proliferation was studied in human promyelocytic leukemia HL-60 cells, which endogenously express AKR1C3 (Ruiz et al., 2011b). It was demonstrated that the pro-proliferative action of AKR1C3 was mediated in part by the RA signaling pathway. Specifically, the addition of lithocholic acid (an AKR1C3 inhibitor) enhanced the antiproliferative effect of 9-*cis*-retinol. The inhibition of AKR1C3 could cause an increased flow in the synthesis of 9-*cis*-RA and, consequently, a lower proliferation rate. The fact that HL-60 cells possess the enzymatic machinery to produce RA, as well as RAR and RXR, makes plausible that the observed effect was due to the action of 9-*cis*-RA. The use of an RAR antagonist restored the proliferation levels obtained with 9-*cis*-retinol alone, confirming that the observed effects were mediated via the RA signaling pathway.

Desmond et al. (2003), by using the same HL-60 model, had previously shown that AKR1C3 inhibition caused cell differentiation and increased synthesis of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15Δ-PGJ_2), a PPARγ ligand. Taken together these results indicate that induced AKR1C3 activity may deplete the agonists of both PPARγ and RAR, resulting in cell proliferation and tumor progression. Therefore, an intriguing possibility arises: AKR1C3 may regulate antiproliferative signaling mediated by heterodimeric complexes between PPARγ and RXR and thus enzyme inhibitors could increase the 9-*cis*-retinol/RA antiproliferative effect, with a potential use in leukemia treatment (**Figure 4**; Ruiz et al., 2011b).

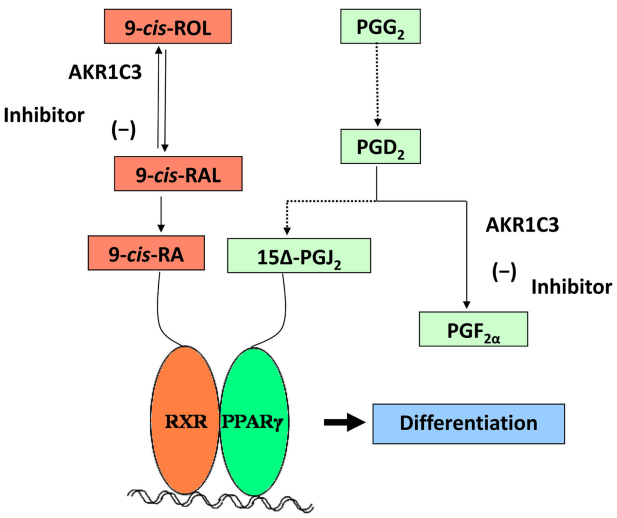

FIGURE 4 | Putative effect of AKR1C3 inhibition in HL-60 cells. A model for AKR1C3 role in leukemia is shown: AKR1C3 inhibition leads to an increase of the 9-*cis*-RA synthesis flow, favoring ligand binding to RAR and RXR. In addition, AKR1C3 inhibition causes an increased synthesis of 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2 (15Δ-PGJ_2), which is the ligand for PPARγ. Activated RXR and PPARγ form heterodimeric complexes and bind to gene regulatory elements. Thus, AKR1C3 inhibition promotes differentiation through these two signaling pathways, inhibiting tumor progression.

In conclusion, accumulated experimental evidence with AKRs and retinoids adds further support to the previous notion that enzymatic activity of AKRs plays a role in the hormonal regulation of cell proliferation at pre-receptor level. In addition, the AKR-mediated modulation of RXR and their ability to form heterodimeric complexes with other nuclear receptors raises the possibility of cross-talk between different signaling pathways. Unbalanced AKR expression may lead to dedifferentiation and increased cancer risk in target tissues, and provides a rationale for the design of AKR inhibitor-based drugs. The structural determinants, here reported, of the unique retinaldehyde specificity of AKR1B10 and 1C3, the most relevant AKRs in cancer generation, may facilitate the finding of selective inhibitors with therapeutic interest.

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The role of human aldo-keto reductases in the metabolic activation and detoxication of polycyclic aromatic hydrocarbons: interconversion of PAH catechols and PAH *o*-quinones

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Polycyclic aromatic hydrocarbons (PAH) are ubiquitous environmental pollutants. They are procarcinogens requiring metabolic activation to elicit their deleterious effects. Aldo-keto reductases (AKR) catalyze the oxidation of proximate carcinogenic PAH *trans*-dihydrodiols to yield electrophilic and redox-active PAH *o*-quinones. AKRs are also found to be capable of reducing PAH *o*-quinones to form PAH catechols. The interconversion of *o*-quinones and catechols results in the redox-cycling of PAH *o*-quinones to give rise to the generation of reactive oxygen species and subsequent oxidative DNA damage. On the other hand, PAH catechols can be intercepted through phase II metabolism by which PAH *o*-quinones could be detoxified and eliminated. The aim of the present review is to summarize the role of human AKRs in the metabolic activation/detoxication of PAH and the relevance of phase II conjugation reactions to human lung carcinogenesis.

Keywords: polycyclic aromatic hydrocarbons, *o*-quinones, aldo-keto reductases, conjugation reactions, phase II metabolism, redox-cycling

INTRODUCTION

The Aldo-keto reductases (AKRs) are a superfamily of monomeric NAD(P)(H)-dependent oxidoreductases. They are cytosolic and have ∼320 amino acids with molecular weights at around 34–37 kDa (Jez et al., 1997). AKRs catalyze the reduction of aldehydes and ketones to yield primary and secondary alcohols on a variety of endogenous substrates and xenobiotics (Hara et al., 1996; Jin and Penning, 2007), and are formal phase I metabolic enzymes. AKRs have been implicated in a number of human diseases. AKR1B1 (aldose reductase) is implicated in the complications that arise due to diabetes, since it converts high blood glucose to the hyperosmotic sugar sorbitol (Lee et al., 1995; Suzen and Buyukbingol, 2003). AKR1B10 (retinal reductase) is involved in retinoic acid signaling and is implicated in the pathogenesis of lung cancer (Fukumoto et al., 2005; Penning and Lerman, 2008) and hepatocellular carcinoma (Liu et al., 2012). By contrast, AKR 1C family members play essential roles in metabolism of male and female sex hormones and may play roles in the development of hormone dependent malignancies of the prostate and breast (Penning and Byrns, 2009); while AKR1D1 (steroid 5β-reductase) is essential for bile-acid biosynthesis and inherited mutations in the *AKR1D1* gene are associated with bile-acid deficiency (Lemonde et al., 2003); The present review will focus on roles of AKRs in metabolism of polycylic aromatic hydrocarbons (PAH).

Polycylic aromatic hydrocarbons are ubiquitous environmental pollutants. They are suspect lung carcinogens and are products of tobacco smoke and incomplete fossil fuel combustion (Grimmer and Bohnke, 1975; Burczynski et al., 1999). PAH are characterized by the presence of two or more fused non-hetero aromatic rings arranged in various configurations (Fetzer, 2007). Based on the arrangement of their aromatic rings, PAH can be categorized into non-bay-region (e.g., naphthalene), bay-region (e.g., benzo[*a*]pyrene), and fjord-region (e.g., benzo[*g*]chrysene) PAH (**Figure 1**). Based on the number of the aromatic rings, the common PAH can be divided into the naphthalene (two rings), phenanthrene (three rings), chrysene, and 5-methyl-chrysene (four rings), benzo[*a*]pyrene (B[*a*]P), and benzo[*g*]chrysene (five rings) series, etc. Benzo[*a*]pyrene is a representative PAH and widely used to study the mutagenic and carcinogenic effects of PAH (Conney, 1982; Cavalieri and Rogan, 1995). PAH are not reactive and require metabolic activation to form electrophiles to elicit their deleterious effects, thus they are procarcinogens (Gelboin, 1980). There are three major pathways for the activation of B[*a*]P, which result in the formation of radical cations, diol epoxides, and electrophilic and redox-active *o*-quinones (**Figure 2**). In the present review, we will focus on the *o*-quinone pathway and discuss the role of human AKRs in the formation of the *o*-quinones, the redox-cycling of *o*-quinones to form catechols, and the removal of catechols by conjugating enzymes. For details about the other pathways, readers are referred to previous review papers (Penning et al., 1999; Penning, 2004; Xue and Warshawsky, 2005).

ACTIVATION OF PAH *trans*-DIHYDRODIOLS BY AKRs TO FORM *o*-QUINONES

In the *o*-quinone pathway of PAH activation, the proximate PAH carcinogens, *trans*-dihydrodiols, e.g., B[*a*]P-7,8-*trans*-dihydrodiol, are oxidized by AKRs to yield ketols which spontaneously rearrange to form catechols, e.g., B[*a*]P-7,8-catechol

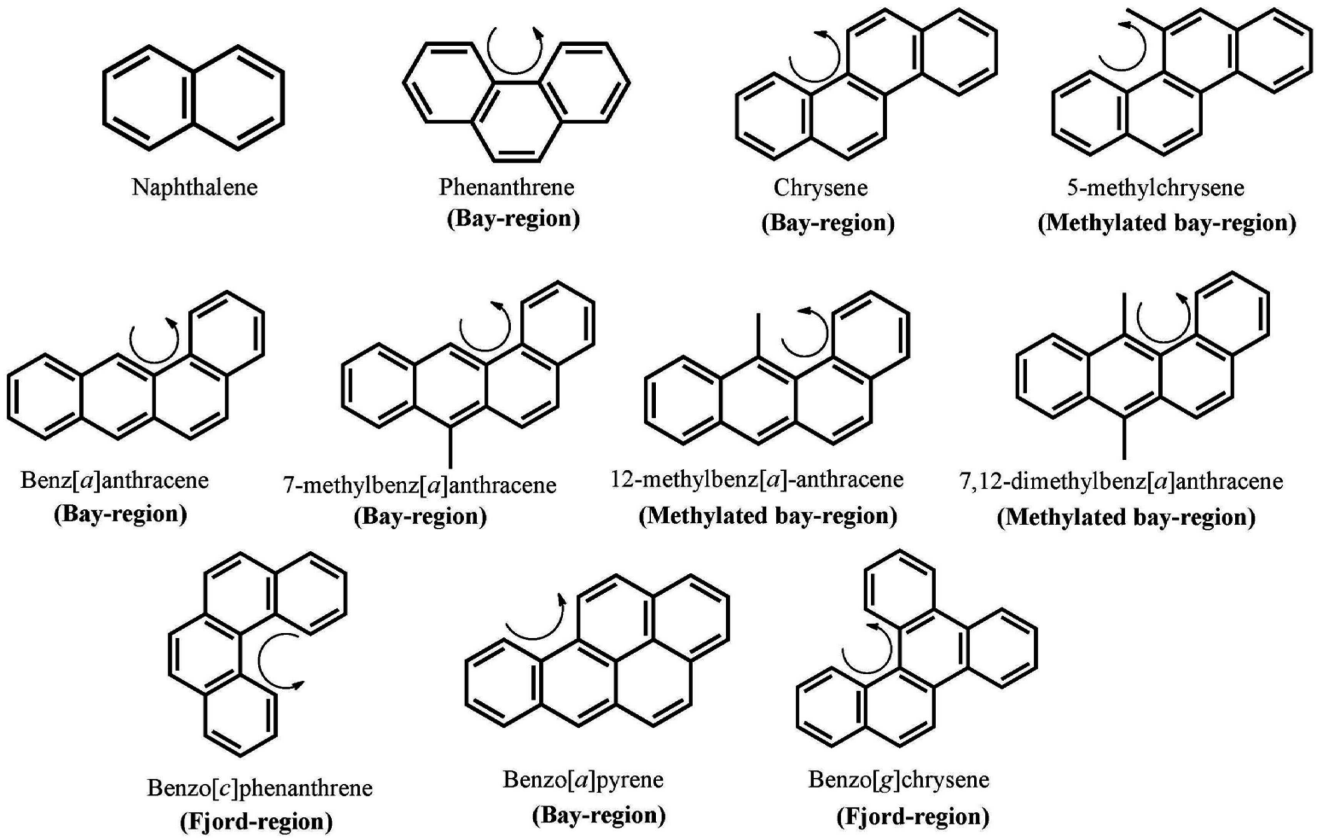

FIGURE 1 | Chemical structures of PAH. The curly arrow denotes the presence of a bay-region; a methylated bay-region or a *fjord*-region.

(**Figure 2**; Burczynski et al., 1998; Palackal et al., 2001, 2002). B[*a*]P-7,8-catechol is not stable and undergoes autoxidation to yield B[*a*]P-7,8-dione. PAH *o*-quinones are electrophilic and highly reactive to endogenous nucleophiles. PAH *o*-quinones can readily form conjugates with cellular thiols to yield L-cysteine, *N*-acetyl-L-cysteine (NAC), and GSH conjugates leading to their elimination (Murty and Penning, 1992a,b). PAH *o*-quinones can also react with DNA to form both stable and depurinating adducts *in vitro* which may result in mutagenesis (Shou et al., 1993; McCoull et al., 1999; Balu et al., 2006). PAH *o*-quinones are also able to undergo non-enzymatic/enzymatic reduction to reform catechols at the expense of NADPH and establish futile redox cycles which amplify the generation of reactive oxygen species (ROS). ROS can cause DNA damage resulting in the formation of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dGuo) lesions, contributing to G-to-T transversions in *K-ras* and *p53* (Kasai et al., 1986; Cheng et al., 1992). PAH *o*-quinones were found to be more mutagenic than diol epoxides in an *in vitro* p53 mutagenesis assay and a linear correlation was observed between the mutagenic efficiency and the presence of 8-oxo-dGuo in the p53 cDNA (Yu et al., 2002; Park et al., 2006; Shen et al., 2006). More recently, the metabolic activation of B[*a*]P-7,8-*trans*-dihydrodiol to B[*a*]P-7,8-dione was demonstrated in human lung adenocarcinoma (A549) cells which shows high constitutive expression of AKRs. This metabolic activation led to the formation ROS and 8-oxo-dGuo lesions in cellular DNA (Park et al., 2008).

Several members of the AKR superfamily are able to oxidize PAH *trans*-dihydrodiols to *o*-quinones (Smithgall et al., 1986, 1988). The substrate specificity of AKRs covers structurally diverse PAH *trans*-dihydrodiols which range from the simplest *trans*-1,2-dihydroxy-1,2-dihydro-naphthalene, to bay-region dihydrodiols (e.g., *trans*-1,2-dihydroxy-1,2-dihydrochrysene), to methylated bay-region dihydrodiols (e.g., *trans*-3,4-dihydroxy-3,4-dihydro-7-methylbenz[*a*]anthracene), and to *fjord*-region dihydrodiols (e.g., *trans*-11,12-dihydroxy-11,12-dihydrobenzo[*g*]chrysene). In contrast, *K*-region dihydrodiols, in which the dihydroxy groups are located on a central benzo-ring, (e.g., *trans*-9,10-dihydroxy-9,10-dihydrophenanthrene and *trans*-4,5-dihydroxy-4,5-dihydroB[*a*]P) are not substrates of AKRs (**Table 1**; Palackal et al., 2001, 2002; Shultz et al., 2008).

In considering the human enzymes, AKR1A1 was stereoselective and will only oxidize (−)-B[*a*]P-7(*R*),8(*R*)-dihydrodiol, which is the major stereoisomer formed *in vivo*. Similarly, AKR1A1 oxidized (−)-benz[*a*]anthracene-3(*R*),4(*R*)-dihydrodiol, (+)-7-methylbenz[*a*]anthracene-3(*S*),4(*S*)-dihydrodiol, and (−)-7,12-dimethylbenz[*a*]anthracene-3(*R*),4(*R*)-dihydrodiol rather than both diastereomers (**Table 1**; Palackal et al., 2001).

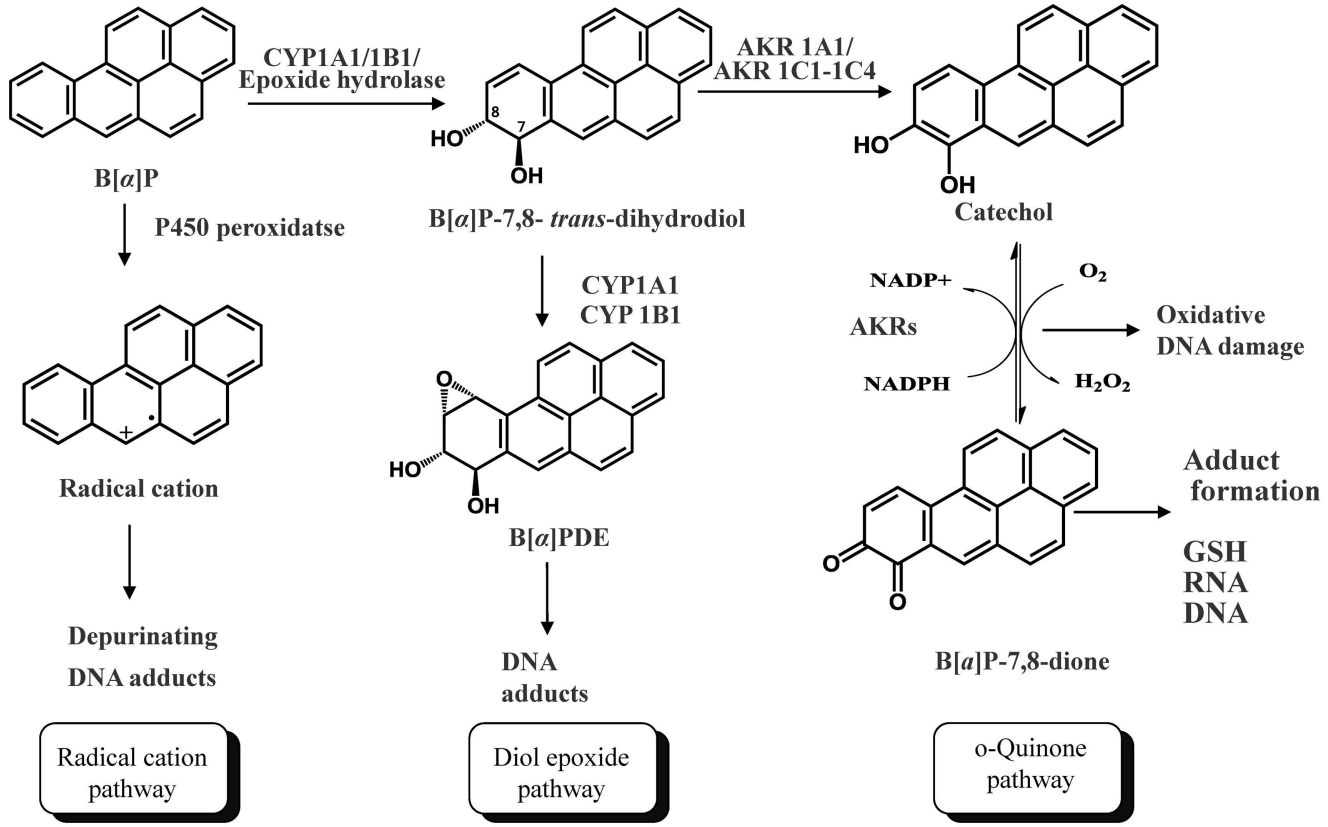

FIGURE 2 | Three Pathways of metabolic activation of PAH and interception by phase II enzymes. (B[*a*]P is used as the representative PAH).

AKR1B10 is one of the most overexpressed genes in non-small lung carcinoma and a member of the smoking gene battery that is up-regulated in response to cigarette smoking and down-regulated in smokers who quit (Fukumoto et al., 2005; Zhang et al., 2008). AKR1B10 was found to oxidize a wide range of PAH *trans*-dihydrodiol substrates *in vitro* to yield PAH *o*-quinones, but showed improper stereospecificity with B[*a*]P-7,8-dihydrodiol in that it only oxidized the minor (+)-B[*a*]P-7(*S*),8(*S*)-dihydrodiol isomer. The related subfamily member AKR1B1 displayed the same stereochemical specificity as AKR1B10 on racemic B[*a*]P-7,8-*trans*-dihydrodiols (Quinn et al., 2008). The stereochemical preference of AKR1B10 appears to be limited only to B[*a*]P-7,8-*trans*-dihydrodiol and benzo[*a*]anthracene-3,4-diol, since no stereospecificity for the oxidation of the (−)-*R,R* and (+)-*S,S* stereoisomers of benzo[*g*]chrysene-11,12-dihydrodiol and 7,12-dimethylbenz[*a*]anthracene-3,4-diol was noted.

Four human AKR1C subfamily members (AKR1C1-AKR1C4) oxidized B[*a*]P-7,8-*trans*-dihydrodiol to B[*a*]P-7,8-dione in the following rank order: AKR1C2 > AKR1C1 ∼ AKR1C3 > AKR1C4 (Burczynski et al., 1998). AKR1C1-AKR1C4 oxidized both stereoisomers of racemic *trans*-dihydrodiols, although AKR1C1 and AKR1C2 displayed a preference for the (+)-B[*a*]P-7(*S*),8(*S*)-dihydrodiol isomer (Burczynski et al., 1998). AKR1C1-AKR1C4 showed high activity for both stereoisomers of the bay-region substituted PAH *trans*-dihydrosiols, where bay-region substituted PAH are more carcinogenic than B[*a*]P (**Table 1**; Palackal et al., 2002).

REDUCTION OF PAH *o*-QUINONES BY AKRs

The metabolic activation of B[*a*]P-7,8-*trans*-dihydrodiol to B[*a*]P-7,8-dione was demonstrated in human lung adenocarcinoma A549 cells which show high constitutive expression of AKRs (Park et al., 2008). This metabolic activation led to the formation of ROS and 8-oxo-dGuo lesions in cellular DNA. Importantly, oxidative stress was exacerbated in the presence of a catechol-*O*-methyl transferase (COMT) inhibitor (Park et al., 2008). This observation indicated that the redox-cycling between B[*a*]P-7,8-dione and B[*a*]P-7,8-catechol occurred with a concomitant generation of ROS which in turn resulted in DNA damage. COMT was able to intercept the catechol and thus protect against the insult from redox-cycling. When B[*a*]P-7,8-dione was given to human bronchoalveolar H358 cells, similar exacerbation of cellular oxidative DNA damage was observed in the presence of a COMT inhibitor (Mangal et al., 2009). Both COMT cell-based studies clearly suggest that two electron reduction of the PAH *o*-quinone to the PAH catechol not only results in oxidative stress and DNA damage, but also leads to *O*-methylation and detoxication of PAH *o*-quinones at the level of PAH catechols (**Figure 2**).

The enzymatic two electron reduction of quinones to hydroquinones is thought to be able to protect against quinone-induced

Table 1 | Oxidation of PAH *trans*-dihydrodiols by human AKRs.

PAH *trans*-dihydrodiols	ARK1A1	ARK1B1	ARK1B10	ARK1C1	ARK1C2	ARK1C3	ARK1C4	AKR7A2	AKR7A3
	k_{cat}/K_m (mM^{-1} min^{-1})								
Naphthalene-1,2-diol	10.4[a]	NA	NA	151[b]	100[b]	6.4[b]	32.7[b]	NA	NA
NON-*K*-REGION DIHYDRODIOLS									
Phenanthrene-1,2-diol	17.9[a]	NA	NA	ND[b]				NA	NA
Chrysene-1,2-diol	15.5[a]	NA	NA	ND[b]	7.03[b]	4.26[b]	10[b]	NA	NA
Benz[*a*]anthracene-3,4-diol	(−) 68.1[a]	NA	(+) 12.8[d]	9.5[b]	17.6[b]	18.0[b]	32.2[b]	NA	NA
Benzo[*a*]pyrene-7,8-diol	(−) 29.6[a]	(+) 10.3[d]	(+) 2.36[d]	22.6[b]	53.3[b]	24.7[b]	16.7[b]	ND[c]	ND[c]
METHYLATED DERIVATIVES									
7-Methylbenz[*a*]anthracene-3,4-diol	(+) 85.8[a]	NA	NA	4.8[b]	49.5[b]	30.1[b]	46.9[b]	NA	NA
12-Methylbenz[*a*]-anthracene-3,4-diol	ND[a]	NA	NA	ND				NA	NA
7,12-Dimethylbenz[*a*]anthracene-3,4-diol	(−) 97.1[a]	NA	2.7[d]	7.4[b]	46.8[b]	19.7[b]	185[b]	NA	NA
5-Methylchrysene-7,8-diol	130[a]	NA	NA	12.4[b]	28.8[b]	9.0[b]	35.2[b]	NA	NA
***K*-REGION DIHYDRODIOLS**									
Phenanthrene-9,10-diol	ND[a]	NA	NA	ND[b]				NA	NA
Benzo[*a*]pyrene-4,5-diol	ND[a]	NA	NA	ND[b]				NA	NA
FJORD-REGION DIHYDRODIOLS									
Benzo[*c*]phenanthrene-3,4-diol	11.8[a]	NA	1.5[d]	ND[b]	4.3[b]	6.6[b]	8.2[b]	NA	NA
Benzo[*g*]chrysene-11,12-diol	11.3[a]	NA	9.55[d]	4.5[b]	4.9[b]	23[b]	165[b]	NA	NA

[a]Palackal et al., 2001, [b]2002, [c]Shultz et al., 2011, [d]Quinn et al., 2008; ND: not detected; + or – in parenthesis, stereospecificity of AKR to the PAH trans-dihydrodiols where no parenthesis exist the AKR isoform oxidizes both isomers of the racemic mixture.

cellular oxidative stress, because the hydroquinone would be available for phase II conjugation reactions. However, if the rates of conjugation reactions are overwhelmed by the rate of the ensuing redox-cycling, the reduction process may be deleterious (**Figure 2**). It is not well understood which enzymes account for the process of two electron reduction of PAH *o*-quinones to PAH catechols and contribute to redox-cycling. Candidate enzymes that may catalyze this reduction include NAD(P)(H):quinone oxidoreductase (NQO1), carbonyl reductases (CBR1 and CBR3), and AKRs. In order to identify the enzymes responsible for the reduction of the PAH *o*-quinones, the ability of homogeneous recombinant NQO1, CBRs, and AKRs to reduce PAH *o*-quinones were compared (Shultz et al., 2011). Except for discrete *o*-quinones, the rank order of activity was: NQO1 > AKR7A2 > CBRs.

NQO1 is a flavoenzyme that catalyzes two electron reduction of quinones to hydroquinones by using NAD(P)H as an electron donor (Jaiswal et al., 1988). Despite its high *o*-quinone reductase activity, NQO1 did not appear to be the dominant enzyme that catalyzes *o*-quinone reduction in human lung A549 cells since treatment with NQO1 inhibitor, dicumarol did not eliminate the deleterious ROS generated by PAH *o*-quinone redox-cycling (Shultz et al., 2011) suggesting that AKRs and CBRs could be the culprit enzymes.

CBRs are cytosolic, monomeric oxidoreductases that catalyze the reduction of a large number of carbonyl compounds (Wermuth, 1981). Human placental CBR1 (15-hydroxyprostaglandin dehydrogenase/prostaglandin 9-ketoreductase) catalyzed the reduction of the non-*K*-region *o*-quinone such as B[*a*]P-7,8-dione (Jarabak, 1991, 1992). However, studies using purified human recombinant CBR1 showed that the substrate specificity of CBR was quite narrow and it reduced *K*-region *o*-quinones but not the non-*K*-region *o*-quinones which are products of PAH *trans*-dihydrodiol oxidation catalyzed by AKRs (Shultz et al., 2011). This suggests that CBR would not play a critical role in the two electron reduction of PAH *o*-quinones.

AKR1C9 (rat liver 3α-hydroxysteroid/dihydrodiol dehydrogenase) was first found to catalyze the reduction of B[*a*]P-7,8-dione at an unexpectedly staggering rate of 4750 nmol/min/mg which was three orders of magnitude greater than the rate of conversion of B[*a*]P-7,8-*trans*-dihydrodiol to B[*a*]P-7,8-dione catalyzed by the same enzyme (Smithgall et al., 1986; Flowers-Geary et al., 1992). In further studies, a panel of purified human AKRs (AKR 1A1, 1B1, 1B10, 1C1-1C4, 7A2, and 7A3) were shown to catalyze the reduction of B[*a*]P-7,8-dione and other PAH *o*-quinones with the specific activities that were 100–1000 times greater than their respective activities to oxidize the cognate PAH *trans*-dihydrodiol (Shultz et al., 2011). Of all AKRs studied, AKR7A2 is the most efficient enzyme for the reduction of B[*a*]P-7,8-dione (**Table 2**).

The AKRs exhibited different reductase activities on series of PAH *o*-quinones which included phenanthrene, chrysene, pyrene, and anthracene series (Shultz et al., 2011). By comparing the ability of AKRs to reduce B[*a*]P-7,8-dione and their ability to oxidize B[*a*]P-7,8-*trans*-dihydrodiol, it was noted that the AKR with the highest quinone reductase activity on a particular PAH *o*-quinone was not always identical to the AKR isoform with the highest dihydrodiol dehydrogenase activity for the respective PAH-*trans*-dihydrodiol. For example, AKR7A2 and AKR7A3 exhibited the highest specific activities for B[*a*]P-7,8-dione reduction, but failed to catalyze the oxidation of PAH-*trans*-dihydrodiols (Shultz et al., 2011; **Tables 1** and **2**). The two electron reduction of PAH

Table 2 | Reduction of B[*a*]P-7,8-dione by human AKRs.

AKR	B[*a*]P-7,8-dione reduction (nmol/min/mg)
AKR1A1	350
AKR1B1	250
AKR1B10	250
AKR1C1	64
AKR1C2	350
AKR1C3	130
AKR1C4	130
AKR1D1	ND
AKR7A2	1270
AKR7A3	1170

Data from: Shultz et al., 2011.

o-quinones catalyzed by AKRs was demonstrated to lead to futile redox cycles. In each instance, 10 μM PAH *o*-quinone consumed 180 μM NADPH, and the consumption of cofactor was accompanied by a concomitant consumption of molecular oxygen and the production of superoxide anion and hydrogen peroxide (Shultz et al., 2011).

The contribution of individual AKRs to the redox-cycling, if PAH *o*-quinones are in lung, will depend on their levels of expression and catalytic efficiency for each PAH *o*-quinone substrate. Although AKR1A1 catalyzes the most effective oxidation of the major enantiomer of B[*a*]P -*trans*-dihydrodiol *in vivo*, (−)-B[*a*]P-7(*R*),8(*R*)-dihydrodiol (Palackal et al., 2001), it has the lowest quinone reductase activity among all AKRs for most PAH *o*-quinones (Shultz et al., 2011). Also, the expression level of AKR1A1 in normal human bronchoalveolar cells is very low (Jiang et al., 2006; Quinn and Penning, 2008), which implies that AKR1A1 is not critical in the enzymatic reduction of PAH *o*-quinones in the lung.

AKR1B10 has a wide substrate specificity for PAH *o*-quinones and exhibits high catalytic efficiency for PAH *o*-quinones particularly for the chrysene series (Shultz et al., 2011). As it is upregulated in response to tobacco smoke exposure (Fukumoto et al., 2005; Gumus et al., 2008; Zhang et al., 2008), AKR1B10 may play an important role in ROS generation from PAH *o*-quinone redox cycling in lung cells. However, AKR1B1 and AKR1B10 only oxidize the minor isomer (+)-B[*a*]P-7(*S*),8(*S*)-dihydrodiol formed *in vivo* with low catalytic efficiency (Quinn et al., 2008), suggesting that AKR1Bs are not as important in the oxidation of B[*a*]P *trans*-dihydrodiols as other AKRs.

Among all AKRs, AKR1C1-1C3 are generally the most efficient isoforms to catalyze the oxidation of PAH *trans*-dihydrodiols (Palackal et al., 2002). They are able to convert both isomers of racemic PAH *trans*-dihydrodiols formed *in vivo* to *o*-quinones (Palackal et al., 2001). AKR1C1-1C3 also display medium to high specific activities for the reduction of most PAH *o*-quinones tested excluding the anthracene series and dibenzo[*a,c*]-phenanthrene-3,4-dione (Shultz et al., 2011). It was found that the expression levels of AKR1C1-1C3 in A549 cells, though lower than that of AKR1B10, were significant higher than AKR1A1 and AKR7A2, suggesting AKR1C isoforms may also be important in catalyzing redox-cycling of PAH *o*-quinones in the lung (Quinn et al., 2008).

Although the expression of AKR7A2 is low in A549 cells, its superior catalytic efficiency for most of PAH *o*-quinones as well as its capability of reducing dimethylbenz[*a*]anthracene-3,4-dione and benz[*a*]anthracene-3,4-dione which are non-substrates of other AKRs may make it play a role in the reduction of these PAH *o*-quinones in lung (Quinn et al., 2008; Shultz et al., 2011).

DETOXICATION OF PAH *o*-QUINONES BY HUMAN COMT

The observation that ROS generation from PAH *o*-quinone in A549 cells was exacerbated by a COMT inhibitor infers that metastable PAH catechols are formed in lung cells and that these catechols can be intercepted by COMT (Park et al., 2008). COMT is a classical phase II enzyme and catalyzes the transfer of a methyl group from *S*-adenosyl-L-methionine (SAM) to the hydroxyl group of a variety of catechols including catecholamine neurotransmitters and the catechol estrogens (Axelrod and Tomchick, 1958; Axelrod, 1966; Ball et al., 1972). There are two major COMT isoforms in human, the soluble cytosolic form (S-COMT), and the membrane-bound endoplasmic reticulum form (MB-COMT), encoded by a single gene at 22q11.2 (Grossman et al., 1992; Tenhunen et al., 1994). The two isoforms share identical amino acid sequences except that the MB-COMT contains an NH_2 terminal extension of 50 amino acids to serve as a hydrophobic anchor to the membrane (Ulmanen and Lundstrom, 1991). COMT is widely distributed among various organs in the body including lung where high COMT activity was found (Mannisto and Kaakkola, 1999). Except in brain, S-COMT is the predominant form in most tissues (Jeffery and Roth, 1984; Grossman et al., 1985; Tenhunen and Ulmanen, 1993).

Polycylic aromatic hydrocarbons *o*-quinones are structurally related to the estrogen *o*-quinones, which demonstrate similar genotoxic modes of action (Penning et al., 1999; Bolton et al., 2000; Bolton and Thatcher, 2008). The formation and detoxication of estrogen *o*-quinones are well studied and can be used as parallel for the studies of PAH *o*-quinone detoxication. The biotransformation of estrogens such as 17β-estradiol and estrone is primarily catalyzed via P450 pathways to yield the 2-hydroxy and 4-hydroxyl catechol estrogens (Aoyama et al., 1990; Kerlan et al., 1992; Martucci and Fishman, 1993; Shou et al., 1997). Both catechol estrogens can be further oxidized to an estrogen *o*-quinone which could form stable and depurinating DNA adducts (Liehr et al., 1986; Stack et al., 1996; Cavalieri et al., 1997). The detoxication of catechol estrogens can occur by *O*-methylation catalyzed by COMT (Schneider et al., 1984; Dawling et al., 2001).

The detoxication of PAH *o*-quinines by COMT was investigated recently (Zhang et al., 2011). B[*a*]P-7,8-dione was reduced to the catechol by dithiothreitol anaerobically in the presence of *S*-adenosyl-L-methionine and further *O*-methylated by human recombinant COMT (Zhang et al., 2011; **Figure 3**). COMT showed quite a wide substrate specificity and *O*-methylated a series of structurally diverse PAH catechols such as bay-region, methylated bay-region and fjord-region PAH catechols. PAH catechols often formed two isomeric products. For B[*a*]P-7,8-catechols, the two

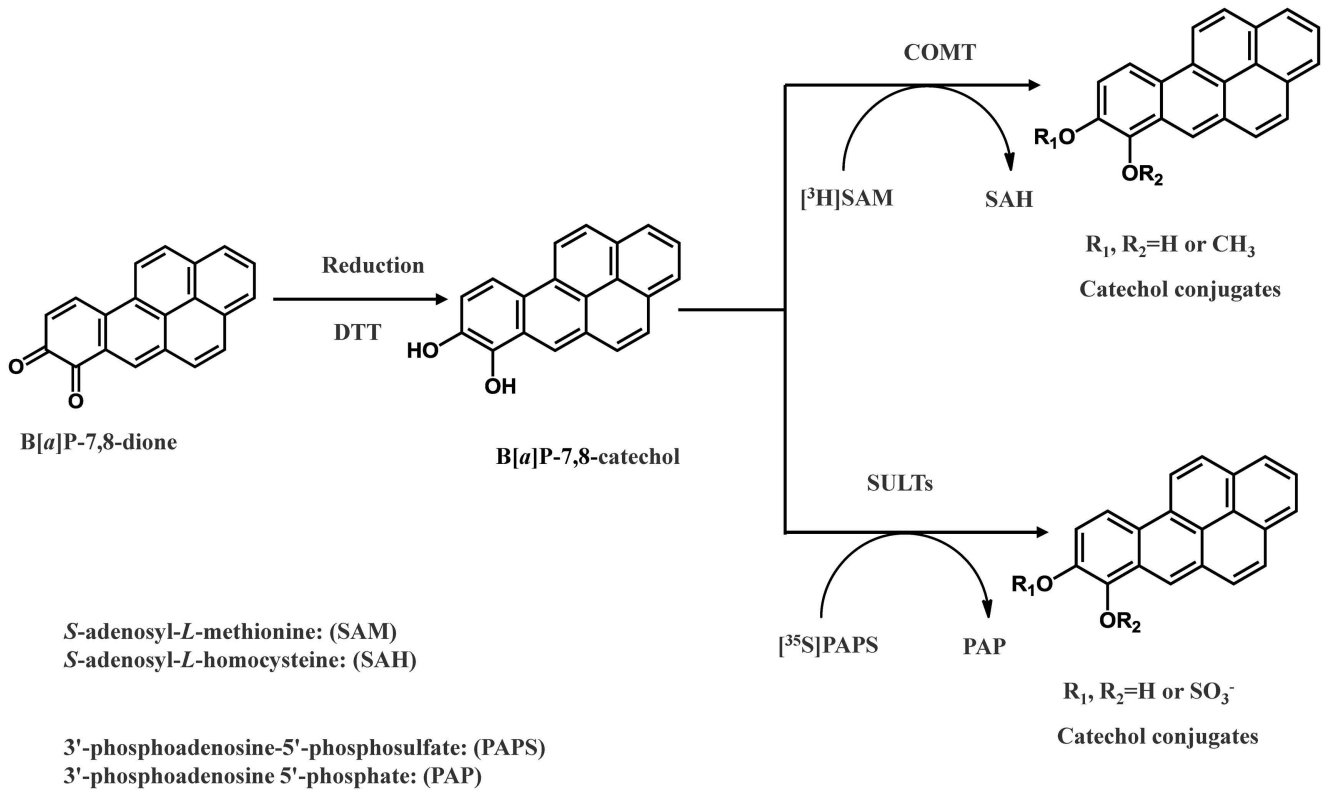

FIGURE 3 | *In vitro* COMT and SULT assay to measure *O*-methylation and *O*-sulfation of PAH *o*-quinones.

products were formed at a ratio of 9:1 with the major metabolite being *O*-8-monomethyl-B[*a*]P-7,8-catechol (Zhang et al., 2011).

Catalytic efficiencies (k_{cat}/K_m) of *O*-methylation of PAH catechols by COMT varied greatly among the classes of PAH catechols studied (**Table 3**). PAH catechols containing a methylated bay-region or a *fjord*-region, which have bent structures due to steric clashing of bay-region hydrogen atoms, have high efficiency of *O*-methylation. However, pronounced substrate inhibition was also observed with these PAH catechols. Since substrate inhibition occurs at low micromolar concentrations, these PAH catechols may not be efficiently detoxified by COMT and thus are more likely to undergo redox-cycling to cause ROS generation (Zhang et al., 2011).

The human *COMT* gene has a common G to A polymorphism that results in valine to methionine substitution at residue 108 for S-COMT or residue 158 for MB-COMT. Compared with the wild type, the Met/Met homozygous COMT activity in red blood cells was reduced by half, and the Met/Val heterozygous COMT showed intermediate activity for 3,4-dihydroxybenzoic acid (Syvanen et al., 1997). The low activity of the COMT mutant is related to its poor thermostability at physiological temperature, and not due to different kinetic properties. This SNP in the *COMT* gene has been associated with an increased risk of lung cancer (Zienolddiny et al., 2008; Cote et al., 2009). As COMT can act as a detoxication enzyme for PAH catechols, it is possible that these polymorphic variants may increase susceptibility to lung cancer caused by PAH.

DETOXICATION OF PAH *o*-QUINONES BY HUMAN SULFOTRANSFERASES AND URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASES

While methylation of estrogen catechols has been found as an important pathway for detoxication of estrogen *o*-quinones, both sulfate and glucuronide conjugates of estrogen catechols catalyzed by the human sulfotransferases (SULTs) and uridine diphosphate glucuronosyltransferases (UGTs), respectively, has been observed (Brueggemeier et al., 1984; Adjei and Weinshilboum, 2002; Taskinen et al., 2003; Hui et al., 2008). Since PAH *o*-quinones are structurally related to the estrogen *o*-quinones, it is very likely that sulfation and glucuronidation of PAH catechols represent other pathways of detoxication of PAH *o*-quinones. SULTs are a group of cytosolic enzymes responsible for the transfer of a sulfonate group from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to either a hydroxyl moiety or an amine group (Negishi et al., 2001). SULTs catalyze the sulfate conjugation of steroid hormones, neurotransmitters, drugs, and xenobiotic compounds (Coughtrie et al., 1998). On the basis of amino acid sequence identity, human SULTs are divided into two main families SULT1 and SULT2, which are also termed the phenol sulfotransferase and the hydroxysteroid sulfotransferase family, respectively. SULT enzymes have a broad tissue distribution including liver, lung, brain, skin, etc. (Cappiello et al., 1989).

In a recent study, it was revealed that SULT1A1, 1A3, and 1E1, were expressed in human lung adenocarcinoma A549 cells, human

Table 3 | *O*-methylation of PAH catechols by COMT.

Quinone	k_{cat}/K_m (min^{-1} μM^{-1})	Substrate inhibition	M1[a] (%)	M2[b] (%)
Naphthalene-1,2-dione	4.9	–	100	0
Chrysene-1,2-dione	1.7	+	62	38
Chrysene-3,4-dione	0.02	–	83	16
5-Methyl-chrysene-7,8-dione	10.1	+	55	45
Benz[*a*]anthracene-3,4-dione	4.0	+	59	41
7-Methylbenz[*a*]anthracene-3,4-dione	1.6	+	53	47
12-Methylbenz[*a*]anthracene-3,4-dione	9.0	+	62	38
7,12-Dimethylbenz[*a*]anthracene-3,4-dione	6.8	+	32	68
Benzo[*c*]phenanthrene-3,4-dione	3.5	–	67	34
B[*a*]P-7,8-dione	0.7	–	90	10
Benzo[*g*]chrsyene-11,12-dione	8.0	+	36	64
Pyrocatechol	0.2	–	NA	NA

a%, Product as isomer 1; b%, Product as isomer 2.

+, Where substrate inhibition is observed; –, substrate inhibition is not observed.

Data from: Zhang et al., 2011.

bronchoalveolar H358 cells, immortalized human bronchial epithelial cells (HBEC-KT), and normal human bronchial epithelial cells (BEAS-2B; Zhang et al., 2012). When B[*a*]P-7,8-dione was reduced anaerobically to the catechol, it was found to be a substrate for these three human recombinant SULTs, and produced two *O*-sulfated products (Zhang et al., 2012). In these assays, the metastable PAH catechol was generated anaerobically and further sulfated by SULTs using PAPS as the sulfate donor (**Figure 3**). Two isomeric mono-*O*-sulfated-B[*a*]P-7,8-catechols were generated and their identities were confirmed by LC-MS-MS and 2D[^{1}H]NMR. The major metabolite formed by SULT1A3 was found to be 8-hydroxy-B[*a*]P-7-*O*-sulfate with the minor metabolite being 7-hydroxy-B[*a*]P-8-*O*-sulfate. SULT1A1 only generated the 8-hydroxy-B[*a*]P-7-*O*-sulfate metabolite. SULT1E1 generated similar amounts of both isomers. SULTs displayed K_m values in the low micromolar or sub-micromolar range which were compatible or even lower than those for estrogen catechols (Zhang et al., 2012). The studies indicate that sulfation of PAH catechols by SULTs could be an important phase II pathway for the detoxication of PAH *o*-quinones, and that the major enzyme involved was SULT1A1.

SULT1A1 polymorphism has been associated with increased lung cancer risk (Wang et al., 2002). The common SULT1A1 allozymes consist of *1 (wild type), *2 variant (Arg213His), and *3 variant (Met223Val; Carlini et al., 2001). The allelic frequencies for SULT1A1*1,*2,*3 in Caucasian were 0.656, 0.332, 0.012, respectively. Despite the low frequency of SULT1A1*3 in Caucasians, it has an allelic frequency of 0.229 in African-Americans (Carlini et al., 2001). It has been reported that SULT1A1 recombinant allozymes have variable thermal stability and specific activity toward *p*-nitrophenol, catechol estrogens, and dietary flavonoids (Raftogianis et al., 1999; Adjei and Weinshilboum, 2002; Nagar et al., 2006). The SULT1A1*2 variant was associated with low enzyme activity and thermal stability (Raftogianis et al., 1999; Wang et al., 2002; Nagar et al., 2006). Although SULT1A1*3 had compatible thermal stability of the wild type, its specific activities for SULT1A1 substrates were lower than that of the wild type in many cases (Nagar et al., 2006). Our study showed that the catalytic efficiency of SULT1A1*3 with B[*a*]P-7,8-catechol was about 50% of the wild type SULT1A1. Therefore, polymorphic variants of SULT1A1 may have reduced efficiency to detoxify PAH *o*-quinones. Unlike the high allelic frequencies of SULT1A1 variants, SULT1A3, and SULT1E1 variants were found to be very rare which suggests that genetic polymorphism of these two enzymes may have minimal effect on PAH *o*-quinone detoxication (Glatt and Meinl, 2004; Hildebrandt et al., 2004).

Uridine diphosphate glucuronosyltransferases are superfamily of microsomal enzymes catalyzing the glucuronidation of a variety of endogenous compounds and xenobiotics (King et al., 2000). Based on sequence identities, UGTs are divided into two main subfamilies, UGT1 and UGT2 (Tukey and Strassburg, 2000). UGTs are widely distributed in a variety of tissues, including liver, intestine brain, kidney, lung, etc. (Guillemette, 2003). Several UGTs were found to catalyze the glucuronidation of PAH mono-phenols and dihydrodiols (Zheng et al., 2001; Olson et al., 2011). The major enzyme isoforms that glucuronidate PAH catechols remain to be identified.

DETOXICATION OF PAH *O*-QUINONES IN HUMAN LUNG CELLS

The existence of Phase II detoxication of PAH *o*-quinones was confirmed in human lung cells (A549, H358, and HBEC-KT cells; Huang et al., 2012b). Consistent with the studies that used human recombinant enzymes, both mono-8-*O*-methylated B[*a*]P-7,8-catechol and mono-8-hydroxy-B[*a*]P-7-*O*-sulfate were formed in three human lung cells. The detection of these metabolites in human lung cells suggests that *O*-methylation and sulfation of PAH catechols are critical pathways in detoxication of PAH *o*-quinones in human lung. Evidence for the formation PAH catechol glucuronides was also found, although absolute chemical structures of them require elucidation.

In addition to the formation of *O*-methylated, *O*-sulfate, and *O*-glucuronide conjugates of B[*a*]P-7,8-catechol, the glutathione (GSH) conjugate, NAC conjugate, and a B[*a*]P-7,8-dione adenine adduct were also detected in human lung cells (**Figure 4**; Huang et al., 2012a,b). PAH *o*-quinones were reported to readily form thioether conjugates with L-cysteine and GSH conjugates *in vitro* (Smithgall et al., 1986; Murty and Penning, 1992a,b). Thio-conjugation occurred at C10 of B[*a*]P-7,8-dione (Murty and Penning, 1992a). The GSH and NAC conjugates of B[*a*]P-7,8-dione formed in the human lung cells were found to be identical to those obtained from non-enzymatic synthesis (Huang et al., 2012b). However, glutathione *S*-transferase (GSTs) may also be involved. To form the NAC conjugate of B[*a*]P-7,8-dione, the GSH conjugate would be converted into a Cys-Gly conjugate by γ-glutamyltranspeptidase, and then further metabolized into a Cys conjugate by the action of a dipeptidase, and ultimately the NAC conjugate would be formed by *N*-acetyl transferase (Blair, 2006, 2010). Future studies will be required to identify the GST isoforms involved in the thio-conjugation of PAH *o*-quinones. Although thio-conjugation of *o*-quinones could enhance the polarity and solubility of PAH *o*-quinones to facilitate the disposition of PAH, the ability of these *o*-quinone thioether conjugates to redox-cycling remains (Monks and Lau, 1997). It was shown that GSH conjugates of benzoquinone undergo redox-cycling to produce renal toxicity. In this respect, thioether conjugates are not completely innocuous. 1,4-Michael addition of PAH *o*-quinones with DNA could also give rise to depurinating and stable DNA adducts. Treatment of lung cells (A549, H358, and HBEC-KT) with 2 μM B[*a*]P-7,8-dione

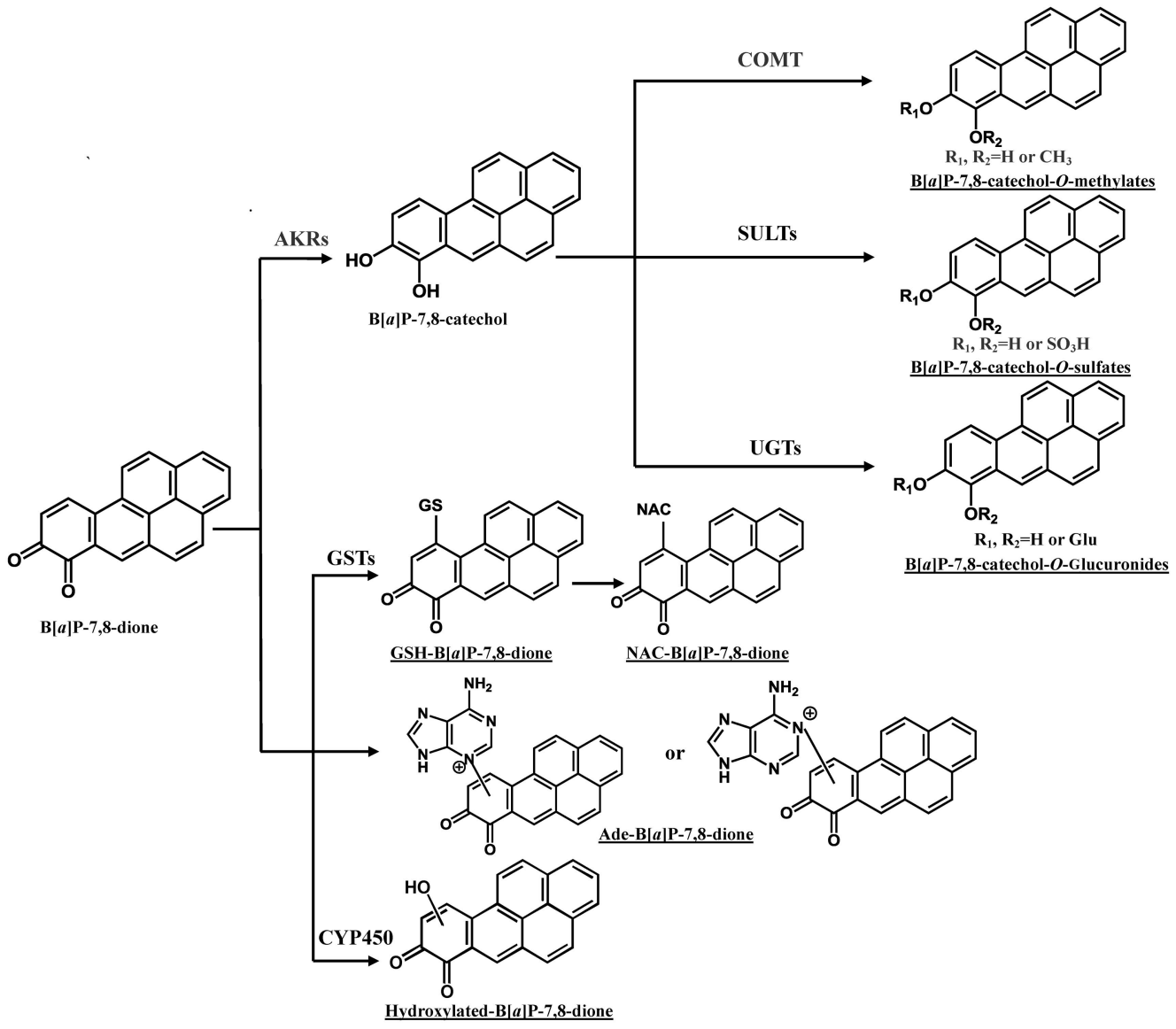

FIGURE 4 | Metabolic pathways of B[*a*]P-7,8-dione in human lung cells. Metabolites of B[*a*]P-7,8-dione detected from human lung cells are underlined (Huang et al., 2012b). Ade, adenosine.

consistently generated a B[*a*]P-7,8-dione adenine adduct (Huang et al., 2012a). Sources of this adduct other than DNA exist, since adenine is a key component of NAD(P)(H) and ATP, and the acid conditions used in its isolation could lead to glycosidic and ester bond cleavage. Thus it is not possible to conclude that this adduct came from DNA.

Our data support the concept that AKRs not only activate PAH *trans*-dihydrodiols by forming redox-active PAH *o*-quinones, but also facilitate the redox-cycling of the PAH *o*-quinones to catechols. The catechols are available for conjugation by a wide range of Phase II enzymes (**Figure 2**). Phase II conjugation of PAH catechols will significantly alter the detrimental properties of PAH *o*-quinone on lung cells. First, it terminates the futile redox-cycling of PAH *o*-quinones that leads to ROS generation and subsequent oxidative DNA damage. Second, it eliminates the electrophilicity of PAH *o*-quinone and prevents the formation of covalent adducts with protein and DNA. Finally, glucuronidation and sulfation usually result in more polar metabolites with enhanced renal or biliary excretion of xenobiotics or drugs, thus conjugation of PAH catechols may also facilitate elimination of PAH *o*-quinones from the body. Since AKRs are involved in activation and deactivation of PAH, it is important to study the expression of AKRs in human lung cells so as to understand the contribution of each AKR isoform in toxification and detoxication of PAH in lung. Except liver-specific AKR1C4, AKR1C1, 1C2, 1C3 were found to be highly expressed in human lung tissue (Penning et al., 2000) were overexpressed in non-small-cell lung carcinoma (Fukumoto et al., 2005; Woenckhaus et al., 2006), and can be induced by PAH (Burczynski et al., 1999; Courter et al., 2007; Misaki et al., 2007; Machala et al., 2008). Transcript levels of AKR1A1, AKR1C, AKR1B, and AKR7A2 isoforms were compared in A549 cells (Quinn et al., 2008). AKR1B1 and particularly AKR1B10 were the most abundant AKR isoforms followed by AKR1C isoforms, while the expression of AKR1A1 and 7A2 were much lower than AKR1B and 1C isoform expression. A549 cells were also found to have significantly greater AKR1B10 transcript levels than found in HBEC-KT cells which are more similar to normal lung epithelium cells. As human lung adenocarcinoma cells, A549 cells may not accurately reflect enzyme levels of normal human lung, further studies are required to investigate expression of the AKRs in cell models that better represent normal human lung cells.

Animal models such as rats and mice are often used to study PAH carcinogenesis raising the possibility that more could be learned by the use of AKR knockout or AKR transgenic mice. However, caution should be exercised when rodent models are used to draw conclusions as to the role of human AKRs in PAH carcinogenesis. The most important enzymes to study would be the murine AKR1C enzymes. However, it has been shown that there are no functional orthologs of the AKR1C enzymes in mice (Velica et al., 2009). Among the studies that have been successful, it was found that AKR1B3 (murine aldose reductase) knockout mice exhibited reduced metabolism of advanced glycation end products (AGEs) resulting in AGEs accumulation and atherosclerotic lesion formation (Baba et al., 2009); in addition AKR1B3 knockout mice prevented azoxymethane-induced formation of colonic preneoplastic aberrant crypt foci by a mechanism that may involve reduction of glutathionyl-4-hydroxynonenal to glutathionyl-1,4-dihydroxynonene (Tammali et al., 2009). In another study, AKR1C18 knockout mice which have 20α-hydroxysteroid dehydrogenase were found to have a parturition defect due to the inability to metabolize progesterone (Piekorz et al., 2005).

CONCLUSION

Aldo-keto reductases catalyze the metabolic activation of structural diverse PAH *trans*-dihydrodiol proximate carcinogens to yield redox-active and electrophilic PAH *o*-quinones. AKRs also catalyze the two electron reduction of PAH *o*-quinones back to the corresponding cognate PAH catechols, establishing a futile redox cycle which results in ROS formation and subsequent oxidative DNA damage in human lung cells. However, the PAH catechols can be intercepted by COMT, SULTs, and UGTs to form conjugated PAH metabolites, which will terminate the redox-cycling. The toxicological outcome of the *o*-quinone pathway of PAH activation will depend on the balance of the activities of the AKR isoforms and the battery of phase II enzymes implicated in the conjugation process. This balance will be affected by gene expression and polymorphic variants of the enzymes identified.

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Enzymes of the AKR1B and AKR1C subfamilies and uterine diseases

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Endometrial and cervical cancers, uterine myoma, and endometriosis are very common uterine diseases. Worldwide, more than 800,000 women are affected annually by gynecological cancers, as a result of which, more than 360,000 die. During their reproductive age, about 70% of women develop uterine myomas and 10–15% suffer from endometriosis. Uterine diseases are associated with aberrant inflammatory responses and concomitant increased production of prostaglandins (PG). They are also related to decreased differentiation, due to low levels of protective progesterone and retinoic acid, and to enhanced proliferation, due to high local concentrations of estrogens. The pathogenesis of these diseases can thus be attributed to disturbed PG, estrogen, and retinoid metabolism and actions. Five human members of the aldo-keto reductase 1B (AKR1B) and 1C (AKR1C) superfamilies, i.e., AKR1B1, AKR1B10, AKR1C1, AKR1C2, and AKR1C3, have roles in these processes and can thus be implicated in uterine diseases. AKR1B1 and AKR1C3 catalyze the formation of PGF2α, which stimulates cell proliferation. AKR1C3 converts PGD2 to 9α,11β-PGF2, and thus counteracts the formation of 15-deoxy-PGJ2, which can activate pro-apoptotic peroxisome-proliferator-activated receptor γ. AKR1B10 catalyzes the reduction of retinal to retinol, and thus lessens the formation of retinoic acid, with potential pro-differentiating actions. The AKR1C1–AKR1C3 enzymes also act as 17-keto- and 20-ketosteroid reductases to varying extents, and are implicated in increased estradiol and decreased progesterone levels. This review comprises an introduction to uterine diseases and AKR1B and AKR1C enzymes, followed by an overview of the current literature on the *AKR1B* and *AKR1C* expression in the uterus and in uterine diseases. The potential implications of the AKR1B and AKR1C enzymes in the pathophysiologies are then discussed, followed by conclusions and future perspectives.

Keywords: endometrial cancer, cervical cancer, endometriosis, uterine myoma, endometriosis, prostaglandins, estrogens, retinoids

UTERINE DISEASES

Uterine diseases include malignant diseases, such as endometrial cancer and cervical cancer, and benign diseases, which include mainly uterine myoma (*myoma uteri*, *uterus myomatosus*) and endometriosis.

ENDOMETRIAL CANCER

Endometrial cancer is the fourth-most-common cancer in Europe and the sixth-most-common cancer worldwide, with the majority of cases arising in post-menopausal women (Ferlay et al., 2008). For 2010, 303,071 new cases and 77,671 deaths were estimated (Ferlay et al., 2008). Most endometrial cancers are sporadic and only 10% of cases are familiar (Amant et al., 2005; Ryan et al., 2005). The sporadic cases can be divided into two subgroups: type 1, the estrogen-dependent endometrioid carcinomas (80%); and type 2, the poorly differentiated, more aggressive form, which is considered to be estrogen independent (20%; Inoue, 2001). Type 1 endometrial cancer is related to exposure to estrogens (of endogenous or exogenous origins) that is not opposed by progesterone or synthetic progestins. This exposure increases the mitotic activity of endometrial cells, along with the number of DNA replication errors and can lead to somatic mutations that result in a malignant phenotype (Inoue, 2001). Development of endometrial cancer is also associated with a number of risk factors, such as obesity, nulliparity, early age at menarche, late onset of menopause, among others (Wallace et al., 2010). In the last few years, it has also been suggested that inflammation contributes to the initiation and progression of endometrial cancer (Wallace et al., 2010). The prostaglandins (PGs) are mediators of inflammation, and they have important roles in the pathogenesis of endometrial cancer. PGE2 and PGF2α are formed locally in cancerous endometrium, where in autocrine/paracrine manners, they can stimulate cell

Abbreviations: 5α-DHP, 5α-dihydroprogesterone; ADH, alcohol dehydrogenase; AKR, aldo-keto reductase; ALDH, aldehyde dehydrogenase; AP1, activator protein 1; COX-2, cycloxygenase-2; FA, farnesoic acid; FAL, farnesal; FOH, farnesol; FT, farnesyltranserase; GABA, γ-aminobutyric acid; GGA, geranylgeranoic acid; GGAL, geranylgeranial; GGOH, geranylgeranyol; GGT, geranylgeranyltransferase; GTPases, guanine nucleotide triphosphatases; IL, interleukin; MAPK, mitogen-activated protein kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; PCLY, prenylcystein lyase; PG, prostaglandin; PKC, protein kinase C; PLC, phospholiase C; PPARγ, peroxisome-proliferator-activated receptor γ; RARs, retinoic acid receptors; RXRs, retinoid X receptors; TNF, tumor necrosis factor.

proliferation, cell adhesion, cell migration, and angiogenesis (Sales et al., 2005, 2008; Wallace et al., 2010).

CERVICAL CANCER

Cervical cancer is the seventh-most-common cancer in women in Europe and the third-most-common cancer worldwide. The majority of cases are detected in developing countries, where it accounts for 13% of all female cancers (Ferlay et al., 2008). For 2010, 553,119 new cases, and 288,109 deaths were estimated as due to cervical cancer (Ferlay et al., 2008). The average age at onset is 45–55 years. The pathology of cervical cancer is related to infection with human papillomavirus (HPV) the DNA of which has been detected in 90% of cervical cancer cases (reviewed in Faridi et al., 2011). HPV, especially high-risk HPV16 and HPV18, are the vectors that confer susceptibility to neoplastic conversion or that directly induce malignant phenotypes in the infected epithelial cells by their oncoproteins E6 and E7, which block the normal functions of the tumor suppressor genes p53 and the retinoblastoma protein (pRb; reviewed in Faridi et al., 2011). Cervical cancer is also related to chronic inflammation of the cervix, due to cellular and molecular changes that can be triggered by human semen, which increase the concentration of cytokines and chemokines. Higher levels of PGE2 are considered as possible promoters of cervical carinogenesis (Herfs et al., 2009). Some studies have shown that risk of cervical cancer increases with use of oral contraceptives, and recently it has been reported that estrogens can promote HPV-induced carcinogenesis (Chung et al., 2010).

UTERINE MYOMAS

Uterine myomas are also known as leiomyomas or uterine fibroids (*myoma uteri* or *uterus myomatosus)*. These are benign tumors of the myometrium that occur in up to 70% of women of reproductive age (Maybin et al., 2011). As such, they are the most common tumors of women of reproductive age, although they have clinically significant symptoms in only one-third of the affected population (Miller, 2008). The majority of cases are thus asymptomatic; when symptomatic, these myomas can be linked to heavy menstrual bleeding, anemia, and even pregnancy complications, which can include difficulty conceiving and increased risk of miscarriage (Miller, 2008). Myomas appear after menarche and decline after menopause, which implicates estrogens as the primary factor that drive their growth. The pathophysiology of uterine myomas is far from clear. At the molecular level, several pathways were associated with this disease, including the retinoic-acid pathway, growth-factor signaling, and extracellular-matrix formation (Zaitseva et al., 2007). Recently, myomas have also been related to aberrant inflammation (Maybin et al., 2011).

ENDOMETRIOSIS

Endometriosis is a complex, estrogen-dependent disease that is defined as the presence of endometrial glands and stroma outside the uterine cavity (Giudice and Kao, 2004). It is diagnosed mainly in women of reproductive age, and estimates show that up to 15% of all pre-menopausal women, and 35–50% of women with infertility and pelvic pain are affected (Giudice and Kao, 2004). Ectopic endometrial tissue can be found in different parts of peritoneal cavity, thus forming three different entities: ovarian, peritoneal, and deep infiltrative endometriosis (Nisolle and Donnez, 1997). The pathogenesis of endometriosis is very complex and remains not completely understood. The most widely accepted is the theory on retrograde menstruation and disturbed immune system (Berkley et al., 2005). However, the pathogenesis also involves changes in apoptosis, cell adhesion, degradation of the extracellular matrix, angiogenesis, cell communication, loss of differentiation capacity (Hompes and Mijatovic, 2007), as well as alterations in other biological pathways (Giudice and Kao, 2004). Also environmental factors, increased local formation of estradiol, and diminished progesterone action affect the development of endometriosis (Giudice and Kao, 2004; Berkley et al., 2005). Enhanced inflammation has been seen in eutopic endometrium of endometriosis patients, and growth of the endometrium in ectopic sites leads to chronic pelvic inflammatory responses, as supported by the increased concentrations of PGE2 and PGF2α in the peritoneal fluid of endometriosis patients (Banu et al., 2009; Lousse et al., 2010). PGE2 regulates proliferation of endometriotic cells, immune suppression, and angiogenesis (Wu, 2005; Wu et al., 2007, 2010), while both PGE2 and PGF2α promote transcription of angiogenic factors, such as vascular epithelial growth factor (Jabbour et al., 2006).

THE ENZYMES OF THE AKR1B SUBFAMILY

There are two well-characterized human members of the aldo-keto reductase 1B (AKR1B) subfamily, AKR1B1 and AKR1B10. These AKR1B enzymes are known as aldose reductases, and they catalyze the reduction of aldehydes to alcohol (glucose to sorbitol in the polyol pathway, and retinal to retinol) and the reduction of a series of other substrates (**Table 1**; Penning and Drury, 2007; Barski et al., 2008).

AKR1B1 is the most studied of the AKRs. The reduction of glucose by AKR1B1 during hyperglycemia has been linked to the development of tissue injury associated with diabetes. Therefore, a number of AKR1B1 inhibitors have been developed for the treatment of diabetic complications (reviewed by Liu et al., 2009; Ramana, 2011; Srivastava et al., 2011; Tammali et al., 2011). In addition to reduction of glucose to sorbitol in the polyol pathway, AKR1B1 catalyzes the reduction of further substrates (**Table 1**), which include lipid peroxidation products, such as 4-hydroxynonenal, and their glutathione (GSH) conjugates, with a higher catalytic efficiency reported for the latter (Barski et al., 2008; Kabututu et al., 2009; Ramana, 2011; **Table 1**). Recombinant AKR1B1 also acts as a PG synthase, as it can convert PGH2 into PGF2α with a lower K_M and a higher V_{max} compared to AKR1C3 (Kabututu et al., 2009). As confirmed by gene silencing, transient transfection studies, and the use of specific inhibitors, AKR1B1 is a functional PGF2α synthase (Bresson et al., 2011). Over the last few years, the involvement of AKR1B1 in inflammatory pathways has also been reported. By reducing GSH conjugated aldehydes, AKR1B1 indirectly stimulates NF-κB, which can lead to activation of the inflammatory cytokines and the inflammatory mediators, such as cycloxygenase-2 (COX-2; Barski et al., 2008; Ramana, 2011; **Figure 1**). AKR1B1 has also been implicated in the development of human cancers, such as liver, breast, ovarian, and cervical cancers (reviewed by Alexiou et al., 2009). It is also associated with resistance toward anticancer drugs, such as the anthracycline antibiotic danorubicin, and cisplatin (Tammali et al., 2011).

Table 1 | Kinetic parameters of the AKR1B1 and AKR1B10 enzymes.

Substrate	AKR1B1			AKR1B10			References
	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	
d,l-Glyceraldehyde	65	33	507	563	29	52	Shen et al. (2011)
4-Hydroxynonenal	716	50	70	31	119	3,839	Shen et al. (2011)
GS-4-hydroxynonanal	5	13	2,600	ND	ND	ND	Shen et al. (2011)
d,l-Glyceraldehyde	50	31	620	5.7	35	6,140	Ruiz et al. (2011a)
All-*trans*-retinal	1.1	0.35	318	0.6	27	45,000	Ruiz et al. (2011a)
All-*trans*-retinol	NA	NA	NA	0.4	4.3	12,750	Ruiz et al. (2011a)
9-*cis*-retinal	ND	ND	ND	0.7	0.9	1,300	Ruiz et al. (2011a)
PGH2	1.9	0.93	491	ND	ND	ND	Kabututu et al. (2009)
PGH2	29	6.06	210	ND	ND	ND	Nagata et al. (2011)
Farnesal	37	27	730	2.5	23	9,100	Endo et al. (2011)
Geranylgeranial	ND	ND	ND	0.9	7.5	8,300	Endo et al. (2011)

NA, no activity; ND, not determined.

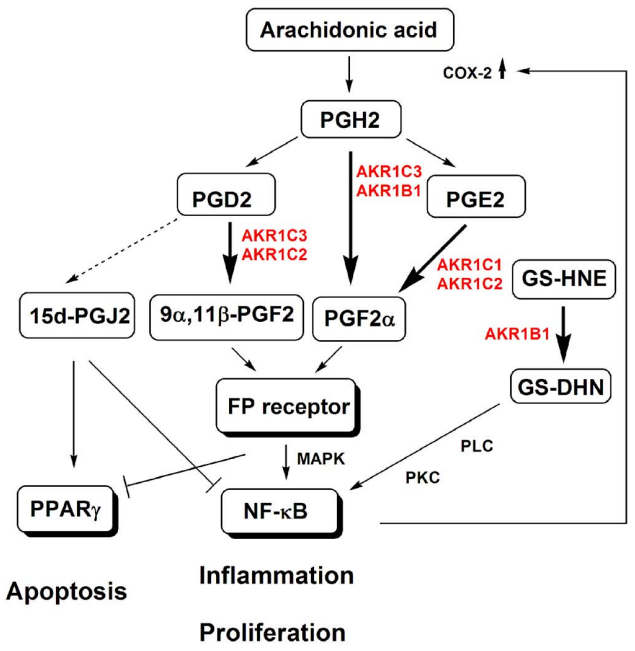

FIGURE 1 | The implications of AKR1B and AKR1C enzymes in prostaglandin biosynthesis and action, and metabolism of lipid peroxidation-derived aldehydes. AKR1B1 reduces lipid-peroxidation-derived aldehydes, such as 4-hydroxynonenal (HNE) and their GSH conjugates, such as the GSH conjugates of HNE (GS-HNE) to GS-DHN. GS-DHN activates the phospholiase C (PLC)/protein kinase C (PKC) pathway and transcription factor NF-κB, which stimulates expression of inflammatory mediators, such as cyclooxygenease-2 (COX-2) and thus leads to pathological inflammation and proliferation. AKR1C3 and AKR1B convert PGH2 to PGF2α, while AKR1C3 and also AKR1C2 convert PGD2 to 9α,11β-PGF2. These two prostaglandins activate the FP receptor, which in turn activates mitogen-activated protein kinase (MAPK) and NF-κB. Additionally, the MAPK inactivate peroxisome proliferator-activated receptor γ (PPARγ), which controls cell growth. By reducing PGD2, AKR1C3 and AKR1C2 prevent the non-enzymatic formation of pro-apoptotic 15d-PGJ2, which would activate PPARγ and inactivate NF-κB.

AKR1B10 has 71% identical amino-acid residues to AKR1B1 and overlapping substrate specificities. With respect to retinols, AKR1B10 has a 100-fold higher catalytic efficiency toward all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal (Barski et al., 2008). AKR1B10 thus counteracts the formation of retinoic acid, a signaling molecule that is involved in the regulation of cell proliferation and differentiation. Similar to AKR1B1, AKR1B10 metabolizes the anticancer agents daunorubicin and idarubicin (Zhong et al., 2011). But in contrast to AKR1B1, which is ubiquitously expressed, AKR1B10 is expressed mainly in the small intestine and colon (Liu et al., 2011), and in different cancerous tissues (hepatocellular, lung, breast, colorectal, cervical, endometrial), although very weakly in normal tissues (Yoshitake et al., 2007). In colon carcinoma and lung carcinoma cells, AKR1B10 silencing induces apoptosis, decreases total phospholipids levels, and increases levels of reductive oxygen species and lipid peroxides (Wang et al., 2009). AKR1B10 also reduces isoprenyl aldehydes and is thus implicated in prenylation of small guanine nucleotide triphosphatases (GTPases) responsible for cell proliferation (Matsunaga et al., 2012; Novelli and D'Apice, 2012). This suggests that AKR1B10 represents an important cell-survival protein and also a novel drug target (Wang et al., 2009). The search for AKR1B10 inhibitors started recently and structurally diverse selective inhibitors of AKR1B10 have already been reported (Endo et al., 2010; Takemura et al., 2011; Soda et al., 2012). AKR1B10 is secreted from normal intestinal epithelium and cancer cell lines through a lysosome-mediated non-classical pathway, which suggests that it also represents a potential serum marker (Luo et al., 2011).

THE AKR1B ENZYMES AND THE UTERUS

The AKR1B1 protein is known to be widely expressed, and the published literature report its expression in normal endometrium and myometrium, where it is induced by IL-1β (Rossi et al., 2005), as well as in the cervix (Saraswat et al., 2006; **Table 2**). As AKR1B1 has a high catalytic efficiency as a PGF2α synthase (Kabututu et al., 2009), it may be responsible for increased production of PGF2α in the secretory as well as the menstrual phases of the

Table 2 | Expression of *AKR1B1* and *AKR1B10* in human uterus.

Isoform	Level	Tissue	References
		Endometrium	
		Epithelial cells **Stromal cells**	
AKR1B1	**mRNA**	Expression related to PGF2α production.	Chapdelaine et al. (2006)
		Induced by IL-1β.	Rossi et al. (2005)
		Expressed throughout the menstrual cycle without significant variations.	Bresson et al. (2011)
		Higher expression in the menstrual phase and middle/late secretory phase.	Catalano et al. (2011)
	Protein	Immunohistochemical staining of luminal and glandular cells, higher expression in early proliferative and mid-late secretory phase of menstrual cycle.	Bresson et al. (2011)
AKR1B10		Not evaluated.	
		Myometrium	
AKR1B1	**mRNA**	Low expression, no significant difference between pregnant and non-pregnant women.	Phillips et al. (2011)
		In cultured cells induced by IL-1β and TNFα.	Phillips et al. (2011)
AKR1B10		Not evaluated.	
		Cervix	
AKR1B1	**Protein**	Detected by immunoblotting and immunohistochemistry.	Saraswat et al. (2006)
AKR1B10		Not evaluated.	

Table 3 | Expression of *AKR1B1* and *AKR1B10* in uterine diseases.

Isoform	Level	Tissue	References
		Endometrial cancer	
AKR1B1		Not evaluated.	
AKR1B10	**Protein**	Staining in 16% cases, 10% to 20% of cells in AKR1B10-positive cases. No correlation with age, clinical stage, pathological features, histological grade, metastasis, and recurrence after surgery.	Yoshitake et al. (2007)
		Cervical cancer	
AKR1B1	**Protein**	Detected in squamous cervical cancer and adenocarcinoma of the cervix, higher protein levels, and enzymatic activity in cancer.	Saraswat et al. (2006)
AKR1B10		Staining in 20% cases, 90% of cells in AKR1B10-positive cases; correlates with tumor recurrence after surgery.	Yoshitake et al. (2007)
		Endometriosis	
AKR1B1		Not evaluated.	
AKR1B10		Not evaluated.	
		Uterine myomas	
AKR1B1		Not evaluated.	
AKR1B10		Not evaluated.	

menstrual cycle, when the highest concentrations of PGF2α have been detected. Concurrently increased synthesis of PGF2α and PGE2 in endometrium has important implications in menstruation. PGF2α acts as a vasoconstrictor and induces myometrial contractions, while PGE2 acts as a vasodilator, which leads to increased edema (reviewed in Maybin et al., 2011). A significantly decreased ratio of PGF2α/PGE2 was observed in women with heavy menstrual bleeding, which might be related to decreased expression/activity of AKR1B1 or increased expression/activity of PGE2 synthase in this condition (reviewed in Maybin et al., 2011). PGF2α has been implicated in the development of primary dysmenorrhea, which again implies a role for AKR1B1 here (reviewed in Maybin et al., 2011). In contrast to AKR1B1, the expression of the *AKR1B10* gene in the endometrium, myometrium, and cervix of normal human uterus has not been reported.

THE AKR1B ENZYMES IN ENDOMETRIAL AND CERVICAL CANCERS

There have been no reports of the expression of *AKR1B1* in endometrial cancer, but AKR1B1 has been detected in squamous cervical cancer and adenocarcinoma of the cervix (Saraswat et al., 2006; **Table 3**). Increased AKR1B1 levels and increased activity detected in cervical cancer (Saraswat et al., 2006) may be related to its implication in inflammation, including its promotion of PGF2α synthesis. It may also be associated with resistance to chemotherapeutics, which is a major problem in treatment of this disease. AKR1B10 has been revealed in cancerous endometrium

and cervical cancer (Yoshitake et al., 2007; **Table 3**). In the later the expression correlated with tumor recurrence after surgery and the authors suggested that AKR1B10 represents a promising marker (Yoshitake et al., 2007). Similarly, as reported in lung cancer (Fukumoto et al., 2005), higher percentages of AKR1B10-positive cells were seen in squamous cell carcinoma (cervical cancer patients), as compared to adenocarcinoma (endometrial cancer patients; Yoshitake et al., 2007). AKR1B10 appears to be specific for this distinct morphology, where it might be associated with reduction of retinal to retinol, and thus with decreased levels of retinoic acid. This will remove the ligands for the retinoic acid receptors (RARs) and retinoid X receptors (RXRs), and thus prevent cell differentiation. At present, there appear to be no reports on the expression of AKR1B1 and AKR1B10 in uterine myomas or endometriosis.

A POTENTIAL ROLE FOR THE AKR1B ENZYMES IN UTERINE DISEASES

AKR1B1 has a relatively high catalytic efficiency as a PGF2α synthase (Kabututu et al., 2009). PGF2α acts through FP receptor and mitogen-activated protein kinase (MAPK) signaling, which activates NF-κB and thus induces COX-2. Higher levels of COX-2 then lead to a further production of the PGs, and thus enhanced cell proliferation, cell adhesion, angiogenesis, and cytoskeleton remodeling of the endometrium (**Figure 1**; Sales et al., 2007, 2008). Additionally, AKR1B1 via reduction of the GSH conjugates of lipid-aldehydes stimulates transcription of various inflammatory cytokines, chemokines, and inflammatory mediators. Lipid-peroxidation-derived aldehydes, such as 4-hydroxynonenal, are conjugated with GSH by GSH-S-transferase and reduced by AKR1B1 to the 1,4-dihydroxy-2-nonene GSH conjugate, which activates PLC, and also NF-κB and AP1 via protein kinase C (PKC; **Figure 1**; Ramana, 2011). Furthermore, the cytokine IL-1β stimulates *AKR1B1* expression (Rossi et al., 2005), which may potentiate the activation of NF-κB and may lead to a vicious inflammatory cycle. The uncontrolled inflammation is related to the development of uterine diseases, including endometrial cancer, uterine myoma, and endometriosis (Wallace et al., 2010; Maybin et al., 2011). As it is involved in inflammation via at least two possible mechanisms, AKR1B1 might also be involved in the pathogenesis of these diseases. To date, higher AKR1B1 expression has been reported only in cervical cancer (Saraswat et al., 2006) and there have been no reports of AKR1B1 expression in endometrial cancer, uterine myoma, and endometriosis. Endometriosis and endometrial cancer are associated with higher PGF2α concentrations in peritoneal fluid and endometriosis tissue, and increased local formation of PGF2α in cancer endometrium, respectively (Sales et al., 2008; Banu et al., 2009), thus AKR1B1 might be implicated in the pathogenesis of these diseases as well. In endometrial cancer cells, PGF2α stimulates cell proliferation, cell adhesion, migration, and angiogenesis (Sales et al., 2008). Also, uterine myoma is related to aberrant inflammation, and thus AKR1B1 might also have a role in this disease.

Due to the high catalytic efficiency of AKR1B10 for the reduction of all-*trans*, 9-*cis*, and 13-*cis* retinaldehydes to retinols (Kabututu et al., 2009), the increased levels of AKR1B10 in endometrial and cervical cancer (Yoshitake et al., 2007) might be linked to depletion of retinoic acid. Decreased availability of the ligand for the RARs and RXRs, will lead to a loss of cell differentiation (**Figure 2**). The oxidation of retinal to retinoic acid by retinaldehyde dehydrogenase is irreversible in a cellular context, and reduction of retinals to retinols by AKR1B10 thus counteracts the synthesis of retinoic acid (Penning, 2005). Indeed retinoic acid and RAR agonists inhibit the growth of the Ishikawa endometrial cancer cell line (Tanabe et al., 2008; Cheng et al., 2011) and retinoic acid stimulates differentiation of the poorly differentiated CAC-1 endometrial adenocarcinoma cell line (Carter, 2003). Also, a very recent report on antitumor effects of inhibitors of the retinoic-acid-metabolizing CYP26A (Goss et al., 2011) confirmed the protective effects of retinoic acid and suggested that AKR1B10 inhibitors might have a similar role. Interestingly, topical retinoic acid has shown activity in cervical intraepithelial neoplasia (a potentially premalignant transformation of cervical cells; Abu et al., 2005), which implies a decreased protective effect of retinoic acid in precancerous tissue. However, in cervical cancer cell lines, no effects (in CaSki cells) and even stimulatory effect (in HeLa cells) of all-*trans* retinoic acid have been observed. As retinoic acid does not repress the expression of the viral oncoproteins E6 and E7 of HPV types 16 and 18, which have a major role in carcinogenesis of the cervix (Myga-Nowak et al., 2011), this shows that retinoids might have a role only in precancerous tissue.

Suppressed action of retinoic acid might also be related to the benign proliferative diseases, such as uterine myoma

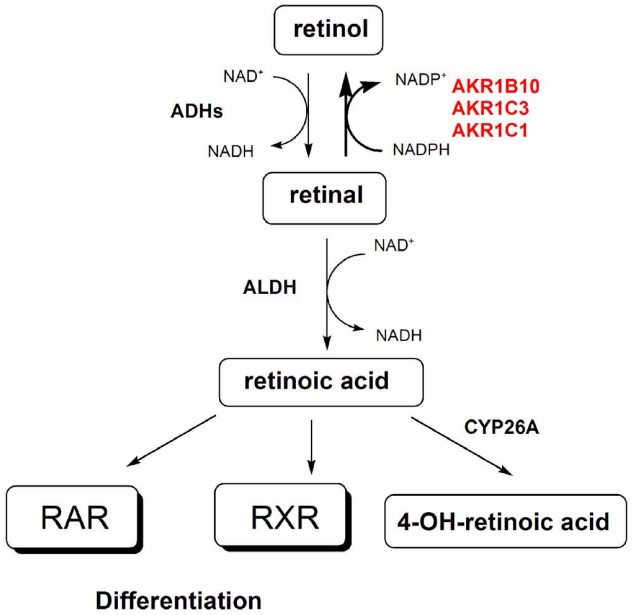

FIGURE 2 | The implications of AKR1B and AKR1C enzymes in retinoid signaling. AKR1B10, AKR1C3, and AKR1C1 catalyze the reduction of all-*trans*-retinal and 9-*cis*-retinal to their corresponding retinols, respectively. The reverse reaction is catalyzed by alcohol dehydrogenases (ADHs). Retinal is further oxidized by aldehyde dehydrogenases (ALDHs) to form retinoic acid, which by binding to the retinoic acid receptor (RAR) and the retinoid X receptor (RXR) stimulates cell differentiation. Retinoic acid is further metabolized by CYP26A to form 4-hydroxy-retinoic acid. Increased levels of AKR1B10 and AKR1C3 might be linked to depletion of retinoic acid, which deprives RAR and RXR of their ligands, and thus blocks cell differentiation.

and endometriosis. Indeed, decreased expression of alcohol dehydrogenase 1 (ADH1) and aldehyde dehydrogenase 1 (ALDH1), which are responsible for the conversion of retinol to retinal and of retinal to retinoic acid, respectively, has been reported in uterine fibroids (Zaitseva et al., 2007). While decreased retinoid uptake, formation and action, and increased retinoic-acid metabolism have been shown in endometriosis (Pavone et al., 2011). It has also been suggested that deficient retinoic-acid action in endometriosis leads to decreased levels of 17β-hydroxysteroid dehydrogenase type 2, which converts the potent estradiol to the less active estrone. This leads to increased levels of the potent mitogen estradiol and thus increased cell proliferation (reviewed in Bulun, 2009). However, evaluation of the expression and role of *AKRB10* in these diseases awaits further studies.

AKR1B10 has a high catalytic efficiency for reduction of isoprenyl aldehydes, farnesal (FAL), and geranylgeranial (GGAL; Endo et al., 2011) and is thus also involved in prenylation of cellular proteins (**Figure 3**). Proteins that undergo prenylation include small GTPases: RAS and RHO proteins (Novelli and D'Apice, 2012). These prenylated proteins are integral components of signaling networks that regulate cell proliferation, differentiation, migration, and apoptosis (Berndt et al., 2011; Novelli and D'Apice, 2012). Prenylation involves transfer of farnesyl pyrophosphate (15-carbon chain) or geranylgeranyl pyrophospate (20-carbon chain), which both originate from the mevalonate pathway, to cysteine residue of these proteins and is catalyzed by farnesyltranserase (FT) and geranylgeranyltransferase 1 (GGT1) and GGT2, respectively. Prenylcystein lyase (PCLY) catalyzes the reverse reaction that releases FAL and GGAL from prenylated proteins (Digits et al., 2002). Farnesyl pyrophosphate and geranylgeranyl pyrophosphate thus serve as substrates for FT and GGT1/GGT2 but can as well be dephosphorylated to form farnesol (FOH) and geranylgeranyol (GGOH; reviewed by Matsunaga et al., 2012). FOH and GGOH are oxidized to FAL and GGAL by ADHs and further to farnesoic acid (FA), geranylgeranoic acid (GGA), and other metabolites. The reduction of FAL and GGAL to FOH and GGOH, catalyzed by AKR1B enzymes, thus recovers substrates for further phosphorylation to corresponding pyrophosphates. Additionally, AKR1B10 with the highest catalytic efficiency for reduction of GGAL prevents formation of GGA and other metabolites with diverse biological effect, including induction of apoptosis (reviewed in Matsunaga et al., 2012). In this manner AKR1B10 prevents formation of pro-apoptotic metabolites and promotes prenylation and activation of small GTPases and further intracellular signaling (reviewed in Matsunaga et al., 2012). Inhibitors of FT (FTI) and GGT1 (GT1I) have already been developed as potential anticancer agents and several clinical trials have been reported for FTI, while the first GGT1I recently entered the clinic (Berndt et al., 2011). FTIs have shown a very limited application in cancer patients, therefore there is a need for novel drug targets within the prenylation pathway, with a potential for AKR1B10. As a secretory protein (Luo et al., 2011), AKR1B10 also represents a potential novel biomarker not only of liver and lung cancer, but also of uterine cancers, and especially of cervical cancer.

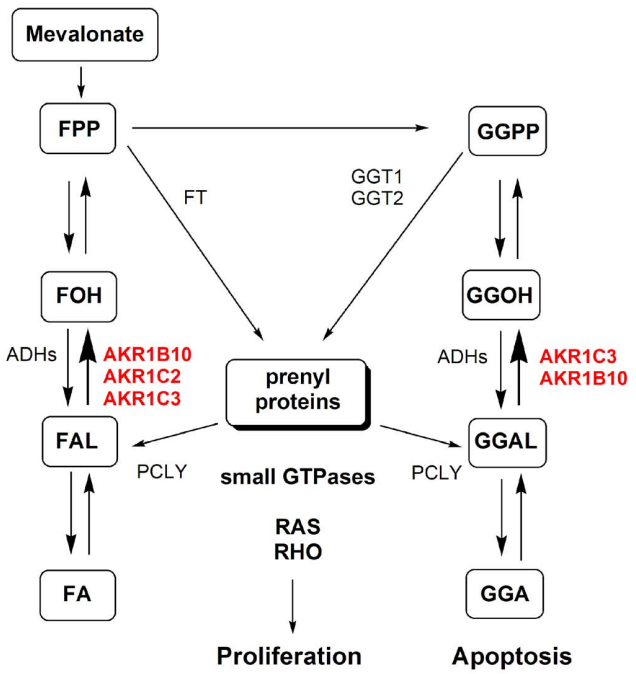

FIGURE 3 | The implications of AKR1B and AKR1C enzymes in protein prenylation. Prenylation involves transfer of farnesyl pyrophosphate (FPP) or geranylgeranyl pyrophosphate (GGPP) by farnesyltranserase (FT) and geranylgeranyltransferase 1 and 2 (GGT1 and GGT2), respectively, to various proteins including small GTPases (RAS and RHO). The reverse reaction that releases farnesal (FAL) and geranylgeranial (GGAL) is catalyzed by prenylcystein lyase (PCLY). Farnesyl pyrophosphate and geranylgeranyl pyrophosphate thus serve as substrates of FT and GGT1/GGT2 but can also be dephosphorylated to form farnesol (FOH) and geranylgeranyol (GGOH). FOH and GGOH are oxidized to FAL and GGAL by alcohol dehydrogenases (ADHs) and by yet unidentified enzymes to farnesoic acid (FA), geranylgeranoic acid (GGA) and other metabolites. The reduction of FAL and GGAL to FOH and GGOH is catalyzed by AKR1B and AKR1C enzymes. This reaction indirectly recovers substrates for further formation of active prenyl-pyrophosphates. Additionally, reduction of GGAL to GGOH prevents formation of GGA and the metabolites with potential apoptotic effects. (Adopted from Endo et al., 2011.)

THE ENZYMES OF THE AKR1C SUBFAMILY

The AKR1C subfamily includes four human enzymes AKR1C1, AKR1C2, AKR1C3, and AKR1C4, which share high percentages of amino-acid identities (84–98%). AKR1C1 and AKR1C2, for instance, differ in only seven amino-acid residues. These enzymes function *in vivo* as 3-keto-, 17-keto-, and 20-ketosteroid reductases to varying extents (**Table 4**), and they thus regulate the activity of androgens, estrogens, and progesterone, and the occupancy and transactivation of the corresponding receptors (Penning et al., 2000; Steckelbroeck et al., 2004). The AKR1C isozymes are also involved in PG metabolism (**Table 4**). AKR1C3 catalyzes the formation of PGF2α from PGH2, and 9α,11β-PGF2 from PGD2, and in this manner prevents the formation of the pro-apoptotic 15-deoxy-PGJ2 (Byrns et al., 2010). AKR1C1 and AKR1C2, on the other hand, convert PGE2 to PGF2α and also have lower 11-ketoreductase activities to reduce PGD2 to 9α,11β-PHF2α (Nishizawa et al., 2000; Dozier et al., 2008). The AKR1C enzymes reduce isoprenyl aldehydes and may be implicated in prenylation of cellular proteins (**Table 4**; Matsunaga et al., 2012). Recently it

Table 4 | Kinetic parameters of the AKR1C1, AKR1C2, and AKR1C3 enzymes

Substrate	AKR1C1			AKR1C2			AKR1C3			References
	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	K_M (μM)	k_{cat} (min^{-1})	k_{cat}/K_M (mM^{-1} min^{-1})	
Progesterone	5.7	0.93	210	ND	ND	ND	2.8	1.04	370	Sharma et al. (2006)
Progesterone	1.9	0.57	300	7.7	0.21	30	5.6	0.168	30	Beranič et al. (2011, 2012)
Estrone	ND	ND	ND	ND	ND	ND	9	0.068	7.6	Byrns et al. (2010)
5α-DHP	1.1	2.6	2,400	0.6	0.48	800	ND	0.06	ND	Higaki et al. (2003)
3α,5α-THP	2.0	4.4	2,200	3.1	0.26	80	ND	ND	ND	Higaki et al. (2003)
20α,5α-THP	0.7	0.6	860	0.5	0.48	960	2.1	0.52	250	Higaki et al. (2003)
PGD2	ND	ND	ND	ND	ND	ND	1.1	1.4	1,270	Matsuura et al. (1998)
PGD2	140	0.015	0.11	120	ND	ND	3	3.7	1,200	Nishizawa et al. (2000)
PGE2	1,400	0.48	0.34	98	0.082	0.83	LA	LA	LA	Nishizawa et al. (2000)
All-*trans*-retinal	LA	LA	LA	NA	NA	NA	1.4	0.60	430	Ruiz et al. (2011b)
9-*cis*-retinal	0.48	0.18	370	NA	NA	NA	0.4	13	32 500	Ruiz et al. (2011b)
Farnesal	3.1	1.7	550	1.1	1.8	1,600	2.6	2.7	1,100	Endo et al. (2011)
Geranylgeranial	ND	ND	ND	ND	ND	ND	0.3	3.6	12,000	Endo et al. (2011)

LA, low activity; NA, no activity; ND, not determined, 3α,5α-THP, 3α-hydroxy-5α-pregnane-20-one; 20α,5α-THP, 20α-hydroxy-5α-pregnane-3-one.

has been reported that AKR1C3 also catalyzes reduction of retinaldehydes, especially 9-*cis*-retinaldehyde with surprisingly higher catalytic efficiency as compared to other substrates (Ruiz et al., 2011b). The AKR1C enzymes act as phase I metabolism enzymes, and they are responsible for metabolism and clearance of different xenobiotics, and are thus implicated in resistance to treatments with different drugs (Huang et al., 2010; Le Calve et al., 2010). The AKR1C enzymes also convert 5α-dihydroprogesterone (5α-DHP; **Table 4**) to the 3α-hydroxy-metabolite, the most potent positive allosteric modulator of the γ-aminobutyric acid type A ($GABA_A$) receptor, as well as to the less potent 20α-hydroxy-metabolite. In this manner AKR1C enzymes are implicated in the production of active neurosteroids. As 5α-pregnanes stimulate proliferation of breast cancer cells, the AKR1C enzymes are also involved in the production of pro-proliferative metabolites (Wiebe, 2006). AKR1C2 preferentially catalyzes the formation of 3α-hydroxy-5α-pregnane-20-one, while AKR1C1 and AKR1C3 mainly form 20α-hydroxy-metabolites (Usami et al., 2002). The AKR1C enzymes, except AKR1C4 which is liver specific, are expressed in different normal and diseased tissues and have thus been related to several diseases, such as lung, breast, prostate, endometrial cancer, myeloid leukemia, and others. Structurally diverse inhibitors have been reported and in spite the high amino-acid identity also selective inhibitors of individual isoforms have been reported (reviewed in Brožic et al., 2011; Byrns et al., 2011).

THE AKR1C ENZYMES AND THE UTERUS

Expression of the *AKR1C* genes has been reported in human uterus (Nishizawa et al., 2000; Penning et al., 2000; **Table 5**). In endometrium, semiquantitative RT-PCR analysis revealed significantly higher mRNA levels of *AKR1C1* in the secretory phase (Nakajima et al., 2003). As the concentrations of PGF2α increase during the secretory phase and PGF2α can activate the *AKR1C1* promoter (Nishizawa et al., 2000), this leads to increased expression of *AKR1C1*. Also mRNA levels of *AKR1C3* are the highest in the early secretory phase (Catalano et al., 2011), while expression of *AKR1C2* throughout the menstrual phases has not yet been examined. To date, expression at the protein/cellular levels has been studied only for AKR1C3 (Pelletier et al., 1999). In paraffin sections of normal endometrium no significant differences in the presence of AKR1C3 were reported during the menstrual phases (Ito et al., 2006). However, these authors provided no information on the specificities of their antibodies. As AKR1C1, AKR1C2, and AKR1C3 have more than 87% identical amino acids, the antibodies might well have detected all of the three isoforms; thus these data should be considered with caution.

The *AKR1C* genes are not expressed only in the endometrium. Expression of *AKR1C1* and *AKR1C3* has been detected also in myometrium (Lee et al., 2008; Phillips et al., 2011; **Table 5**). The authors concluded that the increased *AKR1C1* expression may be responsible for the increased 20α-OHP concentrations that are associated with spontaneous labor, and they suggested that induced labor is not dependent on AKR1C1 (Lee et al., 2008). Expression of the *AKR1C* genes have been detected also in the human cervix (Andersson et al., 2008; **Table 5**). In human cervical fibroblasts IL-1β significantly induced *AKR1C1* and *AKR1C3*, therefore the authors hypothesized that lower uterine infections during pregnancy, with the concomitant production of cytokines, might increase the expression of *AKR1C1* and *AKR1C3* and thus accelerate progesterone metabolism to 20α-OHP in cervical fibroblasts. This would potentially have a major impact on cervical structure, with an increase in the likelihood of a preterm birth (Roberson et al., 2011).

Studies have thus shown that the *AKR1C* genes *AKR1C1* and *AKR1C3* are expressed in the normal uterus within the endometrium, myometrium, and cervical canal (**Table 5**). AKR1C1 is the major human 20-ketosteroid reductase, and

Table 5 | Expression of *AKR1C 1, AKR1C2,* and *AKR1C3* in human uterus

Isoform	Level	Tissue	References
		Uterus	
AKR1C1, AKR1C2, AKR1C3	**mRNA**	*AKR1C2* > *AKR1C3* >> *AKR1C1*	Penning et al. (2000)
		AKR1C3 > *AKR1C1* >> *AKR1C2*	Nishizawa et al. (2000)
		Endometrium	
AKR1C1	**mRNA**	Higher levels in secretory phase.	Nakajima et al. (2003)
		Induced by PGF2α.	Nishizawa et al. (2000)
AKR1C3		Higher levels in early secretory phase.	Catalano et al. (2011)
AKR1C2		Not evaluated.	
AKR1C3	**Protein**	Epithelial cells of endometrial glands.	Pelletier et al. (1999)
		No significant difference between menstrual phases.	Ito et al. (2006)
AKR1C1, AKR1C		Not evaluated.	
		Myometrium	
AKR1C1	**mRNA**	Significantly increased in women in spontaneous labor versus women not in labor, no difference in women with induced labor.	Lee et al. (2008)
AKR1C3		No difference between pregnant/non-pregnant women, no effects of cytokines.	Phillips et al. (2011)
AKR1C2		Not evaluated.	
		Cervix	
AKR1C1, AKR1C2, AKR1C3	**mRNA**	AKR1C1 > AKR1C2 (7-fold) AKR1C1 > AKR1C3 (3-fold) No difference in *AKR1C1* expression in women before/after labor; decreased *AKR1C2* and *AKR1C3* levels during labor.	Andersson et al. (2008)
		Cervical fibroblasts: *AKR1C1* and *AKR1C3* induced by IL-1β.	Roberson et al. (2011)

it converts progesterone to 20α-OHP (Nishizawa et al., 2000; Lanišnik Rižner et al., 2006). AKR1C3 acts as a 17-ketosteroid reductase, and activates estrone to estradiol, while it also acts as a 20-ketosteroid reductase, albeit with 10-fold lower catalytic efficiency than that seen for AKR1C1 (Penning et al., 2000; Beranič et al., 2011). Additionally, AKR1C3 can act as PGF2α synthase (Dozier et al., 2008). AKR1C3 detected in endometrial glands of normal endometrium (Pelletier et al., 1999; Ito et al., 2006) may thus be related to PGF2α formation and progesterone metabolism. The higher levels of *AKR1C1* mRNA in the secretory endometrium are probably associated with increased PGF2α concentrations, and the higher levels of *AKR1C1* mRNA in myometrium, with spontaneous labor (Nakajima et al., 2003; Lee et al., 2008). In late secretory phase endometrium and in myometrium, AKR1C1 thus contributes to decreased local concentrations of progesterone. Induction of *AKR1C1* and *AKR1C3* by IL-1β might be related to preterm labor caused by uterine infection (Roberson et al., 2011). Finally, although AKR1C2 was reported to be the predominate AKR1C isoform of the whole uterus (Penning et al., 2000), its expression and its role in endometrium and myometium have not yet been studied.

THE AKR1C ENZYMES IN ENDOMETRIAL AND CERVICAL CANCER

The expression of the *AKR1C* genes in endometrial cancer has been studied mainly by three groups (**Table 6**). We detected *AKR1C1, AKR1C2*, and *AKR1C3* mRNA levels in paired samples of cancerous endometrium and adjacent control endometrium (Lanišnik Rižner et al., 2006; Šmuc and Lanišnik Rižner, 2009). The differences in expression were not statistically significant, but our pair-wise comparison suggested that in some patients, increased levels of AKR1C1 and/or AKR1C3 might be associated with pathophysiology of endometrial cancer. At the cellular level, Ito et al. (2006) reported increased AKR1C3 immunoreactivity in endometrial hyperplasia and endometrial carcinoma. Although this was without showing any experimental data for the sections or clinical data of the patients, and without sufficient information on the antibodies, the authors suggested that AKR1C3 is one of the key enzymes in the local regulation of estrogen concentrations in endometrial malignancies (Ito et al., 2006). Our group used a well-characterized and specific monoclonal antibody against AKR1C3 (Lin et al., 2004), which stained all 10 paraffin sections of cancerous endometrium (Šmuc and Lanišnik Rižner, 2009). Using the same antibody, Zakhaorov et al. (2010) showed weaker AKR1C3 staining in hyperplastic endometrium and cancerous endometrium, versus the normal proliferative endometrium. However, such comparisons should also be interpreted with caution, due to differences between the groups according to menopausal status, and due to the small number of patients. In contrast to women with normal endometrium (pre-menopausal; mean age, 31.5 years) and patients with endometrial hyperplasia (2 out of 8 post-menopausal; mean age, 47.8), the majority of endometrial cancer patients were post-menopausal (8 out of 12, mean age 58.4), as expected, and there was a significant age difference between the control and the endometrial cancer groups.

Among the *AKR1C* genes, expression of *AKR1C1*, *AKR1C2*, and *AKR1C3* was detected in endometrial cancer and adjacent control endometrium, and increased mRNA levels of *AKR1C1* and *AKR1C3* were seen in some patients (Lanišnik Rižner et al., 2006; Šmuc and Lanišnik Rižner, 2009). However, on average, no significant differences were observed at the mRNA level, and immunohistochemical analysis revealed lower AKR1C3 levels in the hyperplastic and cancer endometrium (Zakhaorov et al., 2010).

Table 6 | Expression of *AKR1C1, AKR1C2,* and *AKR1C3* in uterine diseases

Isoform	Level	Tissue	References
		Endometrial cancer	
AKR1C1, AKR1C2, AKR1C3	**mRNA**	No statistically significant difference (25 paired samples).	Lanišnik Rižner et al. (2006); Šmuc and Lanišnik Rižner (2009)
AK1C3	**Protein**	No statistically significant difference (16 paired samples).	Šmuc and Lanišnik Rižner (2009)
		Increased expression: 50% endometrial hyperplasia, 69% endometrial carcinoma, 19% to 25% normal endometrium samples.	Ito et al. (2006)
		Glandular and luminal epithelial cells, 10/10 paraffin sections of endometrial cancer.	Šmuc and Lanišnik Rižner (2009)
		Weaker staining in endometrial hyperplasia (8) and endometrial cancer (12) versus normal proliferative endometrium (13).	Zakhaorov et al. (2010)
AKR1C1, AKR1C2		Not evaluated.	
		Cervical cancer	
AKR1C1, AKR1C2, AKR1C3	**mRNA**	Increased *AKR1C1* and *AKR1C3* expression in C33A cell line transfected with truncated HPV16E6.	Wanichwatanadecha et al. (2012)
AKR1C1, AKR1C2	**protein**	Detected in 75% HPV positive and 43.2% HPV negative cervical cancer cases, expression correlates with HPV status.	Ueda et al. (2006)
		Endometriosis	
AKR1C1, AKR1C3	**mRNA**	Significantly higher expression in ovarian endometriosis (24) versus control endometrium (10).	Šmuc et al. (2009)
AKR1C1, AKR1C2		Significantly higher expression in ovarian endometriosis (24) versus normal endometrium (10).	Hevir et al. (2011)
AKR1C3	**protein**	In 13/15 samples of ectopic endometrium.	Šmuc et al. (2009)
		No significant difference between ovarian endometriosis (18) and control endometrium (9).	Hevir et al. (2011)
AKR1C1, AKR1C2		Significantly higher levels in ovarian endometriosis.	Hevir et al. (2011)
		Uterine myomas	
AKR1C1, AKR1C2, AKR1C3		Not evaluated.	

It is clear here that AKR1C3 expression in endometrial cancer needs to be further studied at the protein level on a larger number of endometrial cancer samples with the specific antibodies that are available. The menopausal status of the patients should also be taken into consideration, and therefore comparisons between post-menopausal cancer endometrium and control endometrium of the same patient should be used. Additionally, the expression of AKR1C1 and AKR1C2 remain to be studied further.

The AKR1C1 and AKR1C2 enzymes have also been detected in cervical cancer patients with significant correlation to HPV infection and poorer survival rate (Ueda et al., 2006). Interestingly, the up-regulation of *AKR1C1* and *AKR1C3* genes have been reported in cervical cancer C33A cell line stably transfected with truncated HPV16 oncoprotein E6 (Wanichwatanadecha et al., 2012). The authors suggested that the increased levels of AKR1C1 and AKR1C3 may be involved in drug resistance, a major problem in treatment of cervical cancer.

THE AKR1C ENZYMES IN ENDOMETRIOSIS

Our group was the first to show expression of the *AKR1C* genes in endometriosis (**Table 6**). Significantly higher mRNA levels of *AKR1C1*, *AKR1C2*, and *AKR1C3* were seen in ovarian endometriosis versus control endometrium of myoma patients and versus normal endometrium of healthy women (Šmuc et al., 2009; Hevir et al., 2011). At the protein level, AKR1C3 was detected in ectopic endometrium (Šmuc et al., 2009) and immunohistochemical staining showed no significant differences in AKR1C3 scores, but a significant increase in AKR1C2 total scores (Hevir et al., 2011). Although the comparisons to normal endometrium still need to be done, our data suggest that the AKR1C enzymes, and especially AKR1C1 and AKR1C2, may be associated with the development of ovarian endometriosis. Increased levels of AKR1C1 and AKR1C2 in endometriotic tissue might also contribute to enhanced metabolism of the protective progesterone, and to the formation of the pro-proliferative 5α-pregnanes (Wiebe, 2006; Beranič and Lanišnik Rižner, unpublished data). To the best of our knowledge, there have been no reports on the expression of the AKR1C enzymes in uterine myomas.

THE POTENTIAL ROLE OF THE AKR1C ENZYMES IN UTERINE DISEASES

AKR1C3 and AKR1C1 are involved in estradiol formation and progesterone inactivation, respectively (Penning et al., 2000); therefore, their increased levels in diseased endometrium might lead to enhanced proliferation stimulated by estrogens. It has long been known that estrogen actions that remain unopposed by the protective actions of progesterone are related to the development of uterine diseases, endometrial hyperplasia, endometrial cancer, and endometriosis (Inoue, 2001). The increased levels of AKR1C3 that have been seen in some endometrial cancer patients (Lanišnik Rižner et al., 2006; Šmuc et al., 2009) might increase

local concentrations of estradiol and might decrease the concentrations of progesterone, by its 17-ketosteroid and 20-ketosteroid reductase activities, respectively. Increased levels of AKR1C1, the major human 20-ketosteroid reductase, which is also seen in some endometrial cancer patients, might result in enhanced metabolism of progesterone. Also, in ovarian endometriosis, increased levels of AKR1C3 and AKR1C1 might have similar effects on local estradiol and progesterone concentrations. As the growth of uterine myomas is hormone dependent, AKR1C1 and AKR1C3 might also be associated with the development of these benign tumors.

The AKR1C enzymes are involved in the metabolism of 5α-DHP. In breast cancer 5α-pregnanes have been shown to stimulate cell proliferation and detachment (Wiebe, 2006), and also our unpublished studies have confirmed their stimulatory effects on the Z12 endometriotic cell line (Beranič and Lanišnik Rižner, unpublished data). Due to increased levels of 5α-reductase type 1 in endometriosis (Hevir et al., 2011), which catalyzes the formation of 5α-DHP, the AKR1C enzymes might be responsible for enhanced formation of its pro-proliferative 3α/β- and 20α-hydroxy-metabolites. Interestingly, *in vitro* the catalytic activity of the AKR1C enzymes can be blocked by progestins (Beranič et al., 2011, 2012), which are used for the treatment of endometriosis, such as medroxyprogesterone acetate and dydrogesterone. Increased levels of AKR1C1–AKR1C3 and 5α-reductase type 1 in endometriosis might thus lead to enhanced metabolism of progesterone toward the formation of 5α-pregnanes (Hevir et al., 2011). Also, in some endometrial cancer patients, and especially those who are pre-menopausal, and possibly also in patients with myoma, AKR1C1–AKR1C3 may have a similar role.

The AKR1C enzymes are also implicated in PG biosynthesis, forming PGF2α and 9α,11β-PGF2α (Nishizawa et al., 2000; Dozier et al., 2008). These PGs have similar affinities for the FP receptor (Dozier et al., 2008), and both activate this receptor, which exerts diverse responses via the MAPK signaling cascades, including inflammation and proliferation (**Figure 1**). These actions are mediated through the activation of NF-κB and the inactivation of PPARγ. By converting PGH2 to PGF2α and PGD2 to 9α,11β-PGF2, the AKR1C enzymes prevent the formation of the antiproliferative and anti-inflammatory 15d-PGJ2. This PG covalently modifies, and thus activates, PPARγ, while it inactivates NF-κB (Byrns and Penning, 2007). These mechanisms might be used mainly by AKR1C3 but also other AKR1C enzymes in the pathogenesis of cancer endometrium and endometriosis, and possibly also in other uterine diseases.

Similarly as AKR1B enzymes, also AKR1C1 and especially AKR1C3, act as isoprenyl aldehyde reductases (Endo et al., 2011) and retinal reductases (Ruiz et al., 2011b) with higher catalytic efficiencies for GGAL and 9-*cis*-retinal, respectively. As described for AKR1B10 also AKR1C1 and AKR1C3 may be involved in protein prenylation (**Figure 3**) with potential stimulation of proliferation and may also deprive RAR and RXR from their ligand, retinoic acid, which leads to loss of cell differentiation (**Figure 2**). This suggests that inhibitors of AKR1C1 and AKR1C3 would block formation of these prenylated proteins and pro-differentiating retinoic acid. Last but not least, AKR1C enzymes may also be responsible for drug resistance, a frequent and serious problem in treatment of cervical cancer.

CONCLUSION AND PERSPECTIVES

In uterine diseases gene expression and the role of the AKR1C enzymes have been most studied among the enzymes of the AKR1B and AKR1C subfamilies. There are several reports on the expression of *AKR1Cs* in endometrial cancer, cervical cancer, and endometriosis, while there are only two studies on the expression of *AKR1B* in endometrial and cervical cancers. Currently, there are no reports showing the presence of the AKR1B enzymes in uterine myomas and endometriosis, nor of AKR1C in uterine myomas.

AKR1B and AKR1C enzymes are involved in processes that are implicated in the pathophysiology of uterine diseases; therefore, it is clear that these enzymes need to be further investigated. First, we need information on the expression of *AKR1B*s and *AKR1C*s in all parts of the human uterus at the mRNA, protein, and cellular levels, and in diseased versus normal tissues. Secondly, the appropriate model cell lines together with overexpression of individual genes and siRNA approach should be used to delineate the enzymatic activities and the involvement of the salient enzymes in the proposed pathways. As specific inhibitors of these AKR1B and AKR1C enzymes have already been developed these can then be tested in these appropriate cell models.

If further studies reveal the involvement of these AKR1B and AKR1C enzymes in the pathophysiology of uterine diseases, these enzymes would represent potential drug targets. The inhibitors would block the biosynthesis of the proliferative PGF2α (AKR1B1, AKR1C3) and estradiol (AKR1C3), formation of prenylated proteins, which may stimulate proliferation (AKR1B10, AKR1C3), the biosynthesis of 9α,11β-PGF2 (AKR1C3), which deprives pro-apoptotic PPARγ of its ligand, and the biosynthesis of retinol (AKR1B10), which decreases the concentrations of retinoic acid and prevents cell differentiation. These inhibitors might block the proliferative effects and the pathological inflammation in the endometrium, myometrium and cervix, and might thus be efficacious as therapies for women with uterine diseases. Furthermore, there is support for these enzymes, and especially for the secretory AKR1B10, to be used as novel diagnostic markers.

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Role of aldo–keto reductase enzymes in mediating the timing of parturition

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A better understanding of the mechanisms underlying parturition would provide an important step toward improving therapies for the prevention of preterm labor. Aldo–keto reductases (AKR) from the 1D, 1C, and 1B subfamilies likely contribute to determining the timing of parturition through metabolism of progesterone and prostaglandins. Placental AKR1D1 (human 5β reductase) likely contributes to the maintenance of pregnancy through the formation of 5β-dihydroprogesterone (DHP). AKR1C1, AKR1C2, and AKR1C3 catalyze the 20-ketosteroid and 3-ketosteroid reduction of progestins. They could therefore eliminate tocolytic progestins at term. Activation of the F prostanoid receptor by its ligands also plays a critical role in initiation of labor. AKR1C3 and AKR1B1 have prostaglandin (PG) F synthase activities that likely contribute to the initiation of labor. AKR1C3 converts PGH_2 to $PGF_{2\alpha}$ and PGD_2 to 9α,11β-PGF_2. AKR1B1 also reduces PGH_2 to $PGF_{2\alpha}$, but does not form 9α,11β-PGF_2. Consistent with the potential role for AKR1C3 in the initiation of parturition, indomethacin, which is a potent and isoform selective inhibitor of AKR1C3, has long been used for tocolysis.

Keywords: pregnancy, parturition, placenta, myometrium, prostaglandin metabolism, steroid metabolism, aldo–keto reductases

INTRODUCTION

Preterm birth (prior to 37 weeks gestational age) is the principle cause of neonatal morbidity and mortality in the developed world. The United States has one of the highest rates of preterm births in the developed world, occurring in 12–13% of pregnancies (Goldenberg et al., 2008). Preterm deliveries account for 75% of perinatal mortality and surviving preterm infants are at risk for neurological, respiratory, and gastrointestinal complications (Goldenberg et al., 2008; Iams et al., 2008). Treatments such as intravaginal progesterone can be effective at maintaining pregnancies in women at increased risk for preterm labor. Tocolytic therapies to stop active labor do not delay parturition long enough to allow further fetal development, but do provide time for transportation to a hospital with a neonatal intensive care unit (Iams et al., 2008; Mackeen et al., 2011). However, tocolytics have a variety of side effects and there is not enough evidence of benefit to the infant to justify their use (Mackeen et al., 2011).

The mechanisms underlying the initiation of labor are poorly understood, which has limited progress on therapies to maintain pregnancy or stop preterm labor. The lack of a suitable animal model has limited progress toward understanding human parturition (Smith, 2007; Veliça et al., 2009; Hill et al., 2010; Nanjidsuren et al., 2011). The roles of the placenta and other reproductive tissues in parturition, as well as specific steroid and prostaglandin metabolites, differ considerably between mammalian species. Furthermore, the substrate specificities and expression levels of the aldo–keto reductase (AKR) isoforms differ considerably between species. Given the discrepancies in mechanism between species, this review will focus on the AKRs and their substrates and metabolites in human pregnancy.

Progestins play a critical role in human pregnancy, as demonstrated by the efficacy of progesterone in maintaining pregnancy and of the progesterone receptor (PR) antagonist mifepristone in terminating pregnancy and initiating labor (McGill and Shetty, 2007; Iams et al., 2008; Kulier et al., 2011). In other species, a decline in circulating progesterone levels, mediated by distinct pathways, precedes the onset of labor at term (Smith, 2007; Zakar and Hertelendy, 2007). However, serum progesterone levels do not decline during human pregnancies, suggesting that a different mechanism determines the timing of labor. The exact role of progesterone and its metabolites in determining the timing of human labor remains a mystery. Proposed pathways include paracrine regulation through increased myometrial expression of progesterone metabolizing enzymes and/or changes in PR expression. In addition to direct actions of progesterone, actions of its metabolites are likely involved. In particular, 5β-dihydroprogesterone (5β-DHP) inhibits myometrial contractility (Kubli-Garfias et al., 1979; Grazzini et al., 1998; Thornton et al., 1999; Sheehan, 2006). The activities of human AKR1D1 and AKR1Cs suggest they play critical roles in mediating these processes.

Prostaglandins (PG) are also key mediators of parturition. Upregulation of PGH_2 synthase 2 occurs late in pregnancy, resulting in an increase in the synthesis of prostaglandins, particularly the PGF_2 isomers (Mijovic et al., 1999; Slater et al., 1999; Mitchell et al., 2005; Lee et al., 2008b). Activation of the F prostanoid (FP) receptor by prostaglandins stimulates cervical ripening and the initiation of labor (Kelly et al., 2003). AKR1B1 and AKR1C3 are the enzymes that form the PGF_2 isomers in humans.

AKR1D1 IN THE MAINTENANCE OF PREGNANCY

AKR1D1, human 5β-reductase, catalyzes the formation of 5β-androstanes and 5β-pregnanes and contributes to the formation of bile acids in the liver (Charbonneau and Luu-The, 2001; Chen et al., 2011). Of particular relevance to pregnancy, AKR1D1 catalyzes the conversion of progesterone to 5β-DHP (**Figure 1**; Charbonneau and Luu-The, 2001; Chen et al., 2011). 5β-DHP may be a key mediator of the pregnancy maintaining effects of progesterone. While levels of progesterone do not decrease during the final week of pregnancy, levels of 5β-pregnanes decline starting in week 31 (Hill et al., 2007). This decline appears to be largely the result of decreased 5β-reduction, although increased downstream metabolism may also occur. Although 5β-DHP was proposed to bind directly to the oxytocin receptor and antagonize oxytocin binding (Grazzini et al., 1998), others have not been able to replicate this finding (Burger et al., 1999; Astle et al., 2003). 5β-DHP could also inhibit contractions through activation of the pregnane X receptor (Mitchell et al., 2005), allosteric modulation of the GABA receptor (Putnam et al., 1991), or through some as yet unknown mechanism. Although the mechanism is uncertain, it is clear that 5β-DHP limits myometrial contractility; it is significantly more potent than progesterone at inhibiting the contraction of myometrial cells *in vitro* (Kubli-Garfias et al., 1979; Thornton et al., 1999).

Elevated expression of AKR1D1 likely helps maintain human pregnancy. The placenta appears to be the major site of AKR1D1 expression, although it is also expressed in the myometrium (Sheehan et al., 2005). The decline in 5β-DHP levels during labor are accompanied by a significant decline in AKR1D1 mRNA levels. The effect was particularly pronounced in the myometrium, which demonstrated a sevenfold reduction in AKR1D1 levels (Sheehan et al., 2005). Decreased synthesis of relaxatory pregnanes by AKR1D1 may play an important role in the onset of labor.

AKR1D1 exhibits potent substrate inhibition by Δ^4-ene steroids due to binding in a non-productive conformation (Di Costanzo et al., 2008; Faucher et al., 2008; Chen et al., 2011), suggesting a second mechanism for the regulation of its activity. The presence of other Δ^4-ene steroids, particularly 11-deoxycorticosterone and 4-androstene-3,17-dione (Chen et al., 2011), would be anticipated to potently decrease the formation of 5β-DHP by AKR1D1. 4-Androstenedione levels are slightly elevated during labor, while levels of its aromatase product estrone are very high during labor (Hill et al., 2010). Given the permissive ligand binding pocket of AKR1D1 (Di Costanzo et al., 2008; Faucher et al., 2008), it is likely that steroids that are not substrates, including estrogens, will serve as inhibitors. This inhibition might contribute to the induction of labor by estrogens.

Although it is found in higher concentrations than 5β-DHP throughout pregnancy, 5α-DHP formation does not appear to contribute to the timing of labor. 5α-DHP is completely unable to inhibit myometrial contractility *in vitro* (Kubli-Garfias et al., 1979). Ratios of progesterone to 5α-DHP remain constant throughout pregnancy and it appears that expression levels of 5α-reductase enzymes remain elevated throughout pregnancy (Hill et al., 2010). Much of the work that has investigated 5α-reduced pregnanes in pregnancy has focused on their neuroendocrine effects, including their anxiolytic and anesthetic effects in the mother, and their important role in protecting the developing nervous system of the neonate (Amin et al., 2006; Hirst et al., 2006; Hill et al., 2011). Both 5α- and 5β-progestins, particularly allopregnanolone and pregnanolone, act as allosteric modulators of $GABA_A$ receptors (Reddy, 2010).

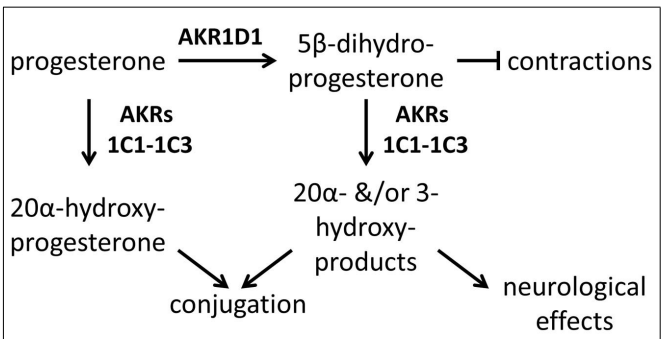

FIGURE 1 | Progesterone metabolism by AKR1D1 and AKR1Cs in pregnancy. AKR1D1 is likely to inhibit myometrial contractions through formation of 5β-dihydroprogesterone, while AKR1C1, AKR1C2, and AKR1C3 are likely to stimulate contractions through the elimination of progesterone and 5β-dihydroprogesterone. AKR1D1 and the AKR1Cs might also contribute to formation of neuroactive progesterone metabolites. The AKR1Cs would have similar activities toward 5α-dihydroprogesterone (not shown).

KETOSTEROID REDUCTION BY AKR1Cs IN PARTURITION

Members of the AKR1C subfamily are likely to help initiate labor by catalyzing the formation of inactive progestin metabolites, leading to paracrine suppression of PR signaling (**Figure 1**). AKR1C1, AKR1C2, and AKR1C3 eliminate progesterone, 5α-DHP, and 5β-DHP through their 20-ketosteroid reductase activities (Penning et al., 2000; Jin et al., 2011). Furthermore, they possess substantial 3-ketosteroid reductase activities that provide another pathway for the metabolism of 5α-DHP and 5β-DHP (Jin et al., 2011). The 20α-, 3α-, and 3β-hydroxy-progestin products of AKR1C enzymes have reduced tocolytic activities and are substrates for elimination through glucuronidation or sulfation. The 3-hydroxy products, such as pregnanolone and allopregnanolone, are neuroactive and could contribute to analgesic and anxiolytic effects in the mother and neuroprotection of the fetus (Steckelbroeck et al., 2004; Amin et al., 2006; Hirst et al., 2006; Reddy, 2010; Hill et al., 2011). The stereochemistry for the reduction of 3-ketosteroids varies between AKR1C isoforms and between 5α-DHP and 5β-DHP, while reduction of the ketone at the 20 position exclusively forms the 20α-stereoisomer (Jin et al., 2011).

AKR1C1, AKR1C2, and AKR1C3 are expressed in reproductive tissues, including the placenta, myometrium, and cervix (Nishizawa et al., 2000; Andersson et al., 2008; Lee et al., 2008a; Hevir et al., 2011). Placental tissues obtained from pregnancies at term reduce progesterone to 20α-hydroxyprogesterone at five times the rate of placentas from the first trimester and there is a further increase in activity with the onset of labor (Milewich et al., 1978; Diaz-Zagoya et al., 1979). It is not known whether expression levels of the AKR1C enzymes correspond to the observed activity. Expression of mRNA encoding AKR1C1 is elevated in

the myometrium during spontaneous, but not oxytocin induced, labor (Lee et al., 2008a). AKR1C1 has the highest catalytic activity toward 20-ketosteroids, and this enzyme likely plays an important role in the inactivation of myometrial progesterone during spontaneous labor (Penning et al., 2000; Jin et al., 2011). Placental expression of AKR1C3 during pregnancy has primarily been examined in the context of its prostaglandin metabolizing effects and is discussed below. AKR1C2 expression in placenta is the lowest of the three peripheral AKR1C isoforms (Nishizawa et al., 2000) and the contribution of AKR1C2 to parturition may be less critical than AKR1C1 and AKR1C3.

Andersson et al. (2008) used intact tissues from combined hysterectomy and cesarean section to examine the role of AKR mediated 20-ketosteroid reductase activity in cervical ripening. They observed mRNA transcripts corresponding to AKR1C1, AKR1C2, and AKR1C3 in cervix, with AKR1C1 exhibiting the highest expression. Cervical tissue from patients in active labor exhibited considerably faster progesterone 20-ketosteroid reduction relative to patients who were not in labor. However, cervical expression of mRNA for the three AKR1C isoforms did not change with labor onset. The increased reductase activity was proposed to result from decreased expression of type 2 17β-hydroxysteroid dehydrogenase, which catalyzes the opposing oxidation reaction (Andersson et al., 2008).

PROSTAGLANDIN SYNTHASE ACTIVITIES OF AKRs

Prostaglandins also regulate parturition. A critical signal in determining the timing of parturition is the release of calcium due to FP receptor activation. Unlike PGE_2, which has receptors that inhibit contractions and others that induce them, $PGF_{2\alpha}$ has only labor promoting effects (Brodt-Eppley and Myatt, 1999). The principle phenotype of FP receptor knockout mice is the inability to deliver young at term, which is rescued by the administration of oxytocin (Sugimoto et al., 1997; Kawamata et al., 2008). A selective FP receptor antagonist inhibits myometrial cell contractility *in vitro* (Friel et al., 2005). Regulation of FP signaling is partially via control of its expression levels and partially through the levels of its PGF_2 ligands, which are determined by the expression of PGH_2 synthase 2, and AKR1B1 and/or AKR1C3 (**Figure 2**; Mijovic et al., 1999; Slater et al., 1999; Mitchell et al., 2005; Lee et al., 2008b; Smith et al., 2011; Watanabe, 2011). Transcript for the FP receptor is suppressed throughout pregnancy, declining with gestational age until immediately before the initiation of labor, when expression levels spike (Brodt-Eppley and Myatt, 1999; Olson et al., 2003). In sheep corpus luteum and rat myometrium, expression of the FP receptor is upregulated by estradiol administration, while progesterone has a suppressive effect, suggesting that steroids may regulate receptor levels during pregnancy (Hoyer et al., 1999; Dong and Yallampalli, 2000).

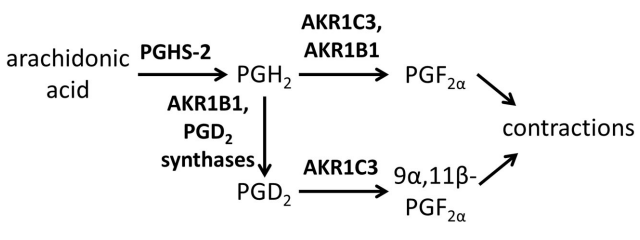

FIGURE 2 | Prostaglandin metabolism by AKR1C3 and AKR1B1 in pregnancy. Both AKR1C3 and AKR1B1 form $PGF_{2\alpha}$ from PGH_2, while AKR1C3 also forms 9α,11β-PGF_2 and AKR1B1 may also contribute to the formation of PGD_2. Both PGF_2 isomers will stimulate myometrial contractions through activation of the FP receptor.

The traditional ligand for the FP receptor is $PGF_{2\alpha}$, although its stereoisomer 9α,11β-PGF_2 is also a potent ligand (Mitchell et al., 2005). Levels of $PGF_{2\alpha}$ in amniotic fluid are low throughout the first 36 weeks of pregnancy before rising during the last few weeks of pregnancy (Lee et al., 2008b). Samples from patients at term indicated a substantial increase in $PGF_{2\alpha}$ from no labor (250 pg/mL) to early labor (640 pg/mL) and advanced labor (4300 pg/mL), which was far greater than the increase in PGE_2 levels. These measurements were performed with a commercial immunosorbent assay with an antibody against $PGF_{2\alpha}$, but cross-reactivity with 9α,11β-PGF_2 was not ruled out. Similar levels of 9α,11β-PGF_2 (400 pg/mL) were detected in amniotic fluid samples from patients undergoing labor at term using an assay with an antibody that had limited cross-reactivity with PGD_2, but not $PGF_{2\alpha}$, suggesting that both isomers may play an important role in labor (Mitchell et al., 2005). Elevated levels of 9α,11β-PGF_2 (200 pg/mL) were detected in patients at term who were not undergoing labor, while levels of this isomer were suppressed in patients prior to 36 weeks, including those undergoing preterm labor. The absence of increased 9α,11β-PGF_2 in preterm labor could be the result of low levels of PGD_2 synthase or low AKR1C3 activity.

The enzymes responsible for the increased synthesis of PGF_2 products at term have not been conclusively identified. However, both of the proposed prostaglandin F synthases are aldo–keto reductases (Smith et al., 2011; Watanabe, 2011). AKR1C3 forms both PGF_2 isoforms. AKR1C3 has substantially higher catalytic activity for the reduction of PGD_2 to 9α,11β-PGF_2 relative to its other endogenous substrates, while the conversion of PGH_2 to $PGF_{2\alpha}$ is also faster than for its steroid substrates (Matsuura et al., 1998; Suzuki-Yamamoto et al., 1999). Involvement of the AKR1B family has only recently been recognized, with the bovine AKR1B5 isoform first shown to synthesize $PGF_{2\alpha}$ (Madore et al., 2003). In humans, AKR1B1 exhibits a higher catalytic activity for the conversion of PGH_2 to $PGF_{2\alpha}$ than AKR1C3, but it is not involved in the formation of 9α,11β-PGF_2 (Kabututu et al., 2009). In addition to synthesizing $PGF_{2\alpha}$, a recent report indicates that in the absence of cofactor, AKR1B1 can catalyze the rearrangement of PGH_2 to form PGD_2; AKR1C3 did not exhibit this activity (Nagata et al., 2011).

AKR1B1 and AKR1C3 are expressed in reproductive tissues during pregnancy and both likely synthesize PGF_2 during pregnancy. Both AKR1B1 and AKR1C3 were cloned based on placental DNA libraries (Grundmann et al., 1990; Dufort et al., 1999). Most of the early work on AKR1B1 focused on its role in regulating glucose metabolism, although it has many additional endogenous substrates (Srivastava et al., 2005). Its roles in prostaglandin signaling have only recently been described (Kabututu et al., 2009; Bresson et al., 2011). AKR1C3 also catalyzes the reduction of a

wide variety of substrates in addition to prostaglandins (Matsuura et al., 1998; Suzuki-Yamamoto et al., 1999; Byrns et al., 2010).

Expression of AKR1B1 and AKR1C3 were recently examined at the mRNA and protein levels in placenta (Breuiller-Fouché et al., 2010). Based on immunohistochemistry, both enzymes were expressed throughout the fetal membranes, but the highest expression was in chorionic trophoblasts and in decidual stromal cells. Western blot and quantitative RT-PCR indicated that AKR1B1 was primarily expressed in the choriodecidua, while AKR1C3 exhibited similar expression levels in both choriodecidua and amnion. Lipopolysaccharide stimulation did not upregulate either protein, suggesting that their expression levels do not contribute to preterm labor induced by intrauterine infection. Changes in placental expression of these enzymes over the course of pregnancy have not been examined.

CONCLUSION

Further research is needed to understand the contribution of the AKRs to the induction of normal labor. Furthermore, very little is known about the roles of the AKRs in mediating signaling during preterm labor, which may be very different than what occurs at term. Evidence supports a role for declining placental and myometrial AKR1D1 expression in the initiation of labor. Increased paracrine inactivation of progestins by AKR1Cs may also be an important step in parturition. Formation of increased levels of PGF_2 isomers stimulates labor, although a major unresolved question is whether changes in the placental expression of AKR1B1 and/or AKR1C3 contribute to this effect. Changes in levels of steroid and prostaglandin products could also result from changes in other enzymes involved in upstream and downstream metabolism.

Although the contribution of AKR1C3 to labor initiation has not been the subject of much research, its enzymatic activities suggest an important role. Each of its enzymatic activities has the potential to contribute to the initiation of parturition. AKR1C3 can eliminate progesterone and its 5α- and 5β-reduced products and can synthesize estradiol and both prostaglandin F_2 isomers (Byrns et al., 2010; Jin et al., 2011). Furthermore, it is potently inhibited by the tocolytic drug indomethacin (Byrns et al., 2008). It is expressed in several reproductive tissues during pregnancy, although it is not known whether expression levels change, except in the cervix where it does not (Nishizawa et al., 2000; Andersson et al., 2008; Breuiller-Fouché et al., 2010). Further investigation into the role of AKR1C3 in parturition is needed.

It remains to be seen whether targeting AKRs can have benefits in pregnancy. AKR1D1 inhibitors might be useful for inducing cervical ripening or given along with oxytocin for the initiation of labor. Inhibitors of the AKR1B and AKR1C enzymes might have benefits for the maintenance of pregnancy or for tocolysis. However, a number of potential pitfalls make developing pharmacotherapies based on AKRs challenging. The lack of an animal model makes testing these targets challenging. Given that mammals have distinct mechanisms of parturition and AKR substrate specificities, it is not clear that effects in animal models will apply to human pregnancies. Another potential issue is that the AKR1C and AKR1D enzymes contribute to the synthesis of neuroactive steroids, such as allopregnanolone and pregnanolone. Given the neuroprotective effects of these steroids, inhibition of these enzymes in the fetal compartment may be undesirable.

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Aldo-keto reductases 1B in endocrinology and metabolism

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The aldose reductase (AR; human AKR1B1/mouse Akr1b3) has been the focus of many research because of its role in diabetic complications. The starting point of these alterations is the massive entry of glucose in polyol pathway where it is converted into sorbitol by this enzyme. However, the issue of AR function in non-diabetic condition remains unresolved. AR-like enzymes (AKR1B10, Akr1b7, and Akr1b8) are highly related isoforms often co-expressed with *bona fide* AR, making functional analysis of one or the other isoform a challenging task. AKR1B/Akr1b members share at least 65% protein identity and the general ability to reduce many redundant substrates such as aldehydes provided from lipid peroxidation, steroids and their by-products, and xenobiotics *in vitro*. Based on these properties, AKR1B/Akr1b are generally considered as detoxifying enzymes. Considering that divergences should be more informative than similarities to help understanding their physiological functions, we chose to review specific hallmarks of each human/mouse isoforms by focusing on tissue distribution and specific mechanisms of gene regulation. Indeed, although the AR shows ubiquitous expression, AR-like proteins exhibit tissue-specific patterns of expression. We focused on three organs where certain isoforms are enriched, the adrenal gland, enterohepatic, and adipose tissues and tried to connect recent enzymatic and regulation data with endocrine and metabolic functions of these organs. We presented recent mouse models showing unsuspected physiological functions in the regulation of glucido-lipidic metabolism and adipose tissue homeostasis. Beyond the widely accepted idea that AKR1B/Akr1b are detoxification enzymes, these recent reports provide growing evidences that they are able to modify or generate signal molecules. This conceptually shifts this class of enzymes from unenviable status of scavenger to upper class of messengers.

Keywords: aldose reductases, adipose tissue, prostaglandins, enterohepatic tissue, metabolism

INTRODUCTION

Aldose reductases (AR) are cytosolic monomeric enzymes, belonging to the aldo-keto reductase (AKR) superfamily. This superfamily encompasses more than 150 NAD(P)(H)-dependent oxidoreductases distributed in all prokaryotic and eukaryotic kingdoms including yeast, plant, invertebrates, and vertebrates. They catalyze the reduction of carbonyl groups from a wide variety of natural or synthetic substrates such as aliphatic and aromatic aldehydes, ketones, keto prostaglandins, ketosteroids, and xenobiotics. Because of overlapping substrates and coenzyme specificities that could lead to confusion between these closely conserved proteins, a nomenclature system for the AKR superfamily has been established based on their structural and genetic properties and is available at www.med.upenn.edu/akr/. Indeed, based on sequence identity, these proteins are divided in 15 families termed AKR1–AKR15, each family having less than 40% amino acid sequence identity with the others. Some families are further subdivided into subfamilies containing proteins with more than 60% sequence identity (Jez et al., 1997; Hyndman et al., 2003). To date, the AKR1 family is the major group encompassing 50 variants of the referring founder protein AKR1A1.

Among the AKR1 family, the AR subgroup (AKR1B) is one of the most characterized because of its involvement in human diseases such as diabetic complications resulting from the ability of AKR1B1 to reduce glucose into sorbitol in a $NADPH + H^+$ dependent manner. In addition to glucose conversion, AKR1B proteins display multiple other activities including reduction of aldehydes generated by lipid peroxidation, steroids and their derivatives or by-products, retinoids, xenobiotics, and prostaglandins.

The AKR1B subfamily contains several proteins with very high structural similarity to the human former AKR1B1. The AKR1B proteins share more than 65% sequence identity (**Table 1**). According to their phylogenetic features and their ability to reduce glucose, they can be classified into two subgroups, i.e., AR (AKR1B1–6) and aldose reductase-like proteins (ARLP; Akr1b7–16), respectively.

To date, three human AKR1B have been characterized: AKR1B1 (human AR; Bohren et al., 1989), AKR1B10 [also designated as human small intestine (HSI) reductase; Cao et al., 1998; Hyndman and Flynn, 1998], and AKR1B15 (Barski et al., 2008; Salabei et al., 2011), which are encoded by genes tandemly arrayed on chromosome 7q33–35. *AKR1B1* seems to be ubiquitously expressed whereas *AKR1B10* expression was only reported in small intestine, colon, liver, and thymus (Cao et al., 1998). A genetic study recently identified a new gene named *AKR1B15* closely related

Table 1 | Comparison of protein sequence of AKR1B.

	% cDNA identity	% Protein identity	% C-ter identity/similarity		Sequences of the 17 C-ter AA residues
HUMAN					
AKR1B1•	100	100	100	100	A**L**LSCTSH**KDYPFH**E**E**F
AKR1B10*	70	71	35	59	NVLQSSHL**EDYPFD**A**E**Y
AKR1B15	68	68	23	47	DFKEFSHL**EDFPFD**A**E**Y
MOUSE					
Akr1b3•	80	85	70	76	A**L**MSCAKH**KDYPFH**A**E**V
Akr1b7^	72	71	52	71	D**L**LDARTE**EDYPFH**E**E**Y
Akr1b8*	71	70	23	53	L**L**PETVNM**EEYPYD**A**E**Y
Akr1b16	69	70	35	71	G**L**FAASHN**EDFPFH**A**E**Y
RAT					
Akr1b4•	82	84	70	76	A**L**MSCAKH**KDYPFH**A**E**V
r-Akr1b10	68	69	35	71	G**L**FAASRN**EDFPFH**S**E**Y
Akr1b13*	76	71	17	53	L**L**PETVNM**EEFPYD**A**E**Y
Akr1b14^	69	68	41	65	G**L**FVTSDE**EDFPFH**E**E**Y

• *or* * *or* ^, *corresponding proteins are encoded by ortholog genes; Bold letter, conserved amino acid residues; AA, amino acid.*

to the *AKR1B1* and *AKR1B10* cluster on chromosome 7, encoding a putative protein sharing 68 and 91% sequence identity with AKR1B1 and AKR1B10, respectively. *AKR1B15* tissue expression has not been explored so far (Barski et al., 2008; Salabei et al., 2011).

Four murine Akr1b have been described: Akr1b3 (murine AR; Gui et al., 1995), Akr1b7 [previously named Mouse vas deferens protein (MVDP); Pailhoux et al., 1990], Akr1b8 [previously named fibroblast growth factor-related protein (FR-1); Donohue et al., 1994], and Akr1b16 (Salabei et al., 2011). Murine AR genes are located on chromosome 6 (locus 6 B1) and their tandem arrangement suggests (as for the three human *AKR1B*) that these four genes arise from an ancestral gene duplication event (Ho et al., 1999; Ruiz et al., 2011). Like *AKR1B1*, *Akr1b3* and *Akr1b16* seem to be ubiquitously expressed (Joshi et al., 2010; Salabei et al., 2011) whereas *Akr1b7* and *Akr1b8* display more restricted tissue distribution: *Akr1b7* is expressed in vas deferens, adrenal glands, gonads, intestine, white adipose tissue, eye, liver, and kidney (Pailhoux et al., 1990; Lau et al., 1995; Tirard et al., 2007; Brunskill et al., 2011; Schmidt et al., 2011). *Akr1b8* expression is detected in testis, heart, adrenal glands, intestine, and liver (Donohue et al., 1994; Lau et al., 1995; Joshi et al., 2010).

Among rat AR, Akr1b4 seems to be ubiquitously expressed whereas the other related proteins have a more restricted expression pattern (MacLeod et al., 2010). Transcripts for *Akr1b14* have been detected in liver, kidney, and adrenals. Those of *r-Akr1b10* share this tissue expression pattern but are also found in brain, heart, and lungs (Endo et al., 2010b). *Akr1b13* expression has been observed at mRNA level in almost all organs, except brain and liver but the protein remains undetectable in small intestine and colon (Endo et al., 2011).

Several studies allowed identification of *Akr1b4*, *Akr1b13*, and *Akr1b14* as the orthologs of mouse *Akr1b3*, *Akr1b8*, and *Akr1b7*, respectively (Barski et al., 2008; Endo et al., 2011). This phylogenetic analysis between rat and mouse AR has some limits: Akr1b14 and Akr1b7 do not exactly display the same expression pattern. Indeed, vas deferens is the major site of Akr1b7 expression whereas Akr1b14 is barely detectable in this tissue. Despite high sequence identity, the two promoters differ by a short 77-bp region absent in rat sequence which confers vas deferens targeted expression and androgen responsiveness to the *Akr1b7* gene (Val et al., 2002).

Identification of the mechanisms regulating expression of AKR1B/Akr1b is a necessary step to understand their physiological functions. Nevertheless, the precise identification and determination of spatial distribution of AKR1B/Akr1b in healthy tissues remains a difficult task, as it requires powerful and specific immunological or nucleic probes to allow discrimination between these closely related isoforms. For that reason, we designed immunological probes targeting the most divergent domain among these enzymes that encompass the 17 C-terminal amino acid residues (**Table 1**). Indeed, using this C-terminal region critical to substrate specificity (Bohren et al., 1992), we developed immunological tools discriminating Akr1b7, Akr1b3, and Akr1b8 isoforms (Lefrançois-Martinez et al., 2004). Similarly, the most discriminating nucleotide probes or primers should be carefully designed from 5′- to 3′-untranslated regions while open reading frame should be avoided whenever possible. Knowing that in mammals, AR genes (i.e., human *AKR1B1* gene, murine *Akr1b3*, and rat *Akr1b4*) display a broad tissue expression pattern, it can be assumed that in normal conditions, most of their activities are involved in maintaining general cellular homeostasis through osmotic regulation or detoxification processes.

This review will focus on the most characterized AKR1B proteins: AKR1B1, Akr1b3, Akr1b7, Akr1b8, and AKR1B10. To date many *in vitro* and *ex vivo* studies have examined and carefully described their enzymatic activities and defined their substrate specificities. These studies have enlightened some redundancy in substrate specificities that can be confusing when considering that various isoforms may coexist in the same tissue. In light of these enzymatic data, one of the most challenging issue regarding

AKR1B enzymes would now be to explore their distinct biological functions in specific physiological or pathological processes. The focus of this review is to integrate most recent data on specific regulations of AR genes with enzymatic and functional data, in selected organs involved in endocrine and metabolic function, i.e., the adrenal gland, enterohepatic tissue, and white adipose tissue.

AKR1B AND ADRENAL ENDOCRINE FUNCTION

The adrenal gland has two anatomically and functionally different components: the outer cortex which provides mineralocorticoids from the *zona glomerulosa*, glucocorticoids from the *zona fasciculate*, and the inner medulla in which chromaffin cells produce adrenal catecholamines, i.e., epinephrine and norepinephrine and various neuropeptides.

Acute and chronic adrenal cortex steroidogenesis is regulated mainly through activation of the cAMP-dependent protein kinase (PKA) signaling pathway mediated by the pituitary adrenocorticotropin hormone (ACTH).

Excess levels of glucocorticoid in the plasma in turn induce a negative feedback on ACTH production resulting in blunted ACTH-dependent steroidogenesis in the adrenal gland. This blockade of the hypothalamic-pituitary-adrenal axis can be experimentally recapitulated by dexamethasone treatment (a synthetic glucocorticoid). In adrenals, cAMP-induced PKA activation results at least in the phosphorylation of transcription factors such as steroidogenic factor 1 (SF-1), CCAAT Enhancer Binding Protein (C/EBP), and cAMP response element-binding protein. These, in turn stimulate transcription of genes encoding steroidogenic enzymes and proteins responsible for cholesterol metabolism, mobilization and transport. Therefore, adrenal steroidogenesis is strongly associated with production of endogenous harmful lipid aldehyde by-products including isocaproaldehyde (4-methylpentanal) derived from cholesterol side chain cleavage (the first step of steroid synthesis) and 4-hydroxynonenal (4-HNE). Interestingly, previous studies have established that the adrenal gland is one of the major sites for human and murine AR expression (Lau et al., 1995; Hyndman and Flynn, 1998).

Akr1b8/AKR1B10: EXPRESSION PROFILE, DETOXIFICATION FUNCTION (TABLES 2 AND 3)

Akr1b8 messenger was observed by *in situ* hybridization analysis in fetal and adult murine adrenal cortex and remained undetected in the medulla (Lau et al., 1995). Nevertheless, dexamethasone-induced ACTH suppression had no effect on Akr1b8 protein levels (Lambert-Langlais et al., 2009) suggesting that its biological function would not be associated to the ACTH-dependent steroidogenic activity. Indeed, isocaproaldehyde reductase activity was abolished in adrenocortical Y1 cells lacking Akr1b7 (due to stable antisense expression) in the presence of unaltered Akr1b8 protein levels (Lefrancois-Martinez et al., 1999). Accordingly, isocaproaldehyde accumulated and resulted in cellular toxicity despite the presence of Akr1b8. On the basis of these functional studies, of its enzymatic constants and constitutive expression, Akr1b8 does not appear to be the major isocaproaldehyde reductase in the adrenal cortex (Martinez et al., 2001). Although all murine AR exhibit 4-HNE reductase activity, Akr1b8 seems to be the most efficient HNE reductase in mouse tissues (Srivastava et al., 1998; Martinez et al., 2001). This suggests that adrenocortical expression of Akr1b8 could be dedicated to detoxification of aldehyde lipids that are present in large amounts in the cortex (Burczynski et al., 1999). Nevertheless, functional demonstration remains to be established.

AKR1B10 mRNA has been detected in adrenal glands using a human RNA Master Blot, but to date, there is no information available on its *in situ* localization and transcriptional control in this organ (Hyndman and Flynn, 1998). Although both AKR1B1 and AKR1B10 human AR display 4-HNE reductase activity, AKR1B10 exhibits a higher activity and product turn over than AKR1B1 (Shen et al., 2011). Both AKR1B1 and AKR1B10 isoforms are expressed in the human adrenal gland and *ex vivo* studies revealed that they also share the ability to reduce isocaproaldehyde (Hyndman and Flynn, 1998). Nevertheless, in a comparative enzymatic study, Hara and colleagues showed that AKR1B1 had a more effective isocaproaldehyde reductase activity than AKR1B10, suggesting that the latter was unlikely to play a major role in the detoxification of steroidogenic by-products (Endo et al., 2009b).

Since its identification in human hepatocellular carcinoma, AKR1B10 was shown to be differentially expressed in other tumor and normal tissues (Cao et al., 1998; Fukumoto et al., 2005; Yoshitake et al., 2007; Rajkumar et al., 2010). AKR1B10 was suggested to be a biomarker of smoker's non-small cell lung carcinomas (Fukumoto et al., 2005) and to be involved in drug resistance (Matsunaga et al., 2012).

Human adrenocortical carcinoma (ACC) are rare malignant tumors generally associated with a poor prognosis (Libè et al., 2007). In order to identify molecular predictors of malignancy and of survival, 153 unilateral adrenocortical tumors were studied by microarray (http://www.ebi.ac.uk/arrayexpress, experiment E-TABM-311). Unsupervised clustering analysis was performed which allowed robust discrimination of malignant and benign tumors. On the basis of this analysis, AKR1B10 was not found to be associated with the ACC group (de Reyniès et al., 2009).

Akr1b7: EXPRESSION PROFILE, DETOXIFICATION FUNCTION, AND PARACRINE ACTION (TABLES 2 AND 3)

High levels of *Akr1b7* transcripts were initially observed by *in situ* hybridization in fetal and adult murine adrenal cortex but were undetectable in the medulla (Lau et al., 1995). We confirmed these results by immunohistochemistry experiments which allowed us to further restrict Akr1b7 expression to the *zona fasciculata* (Aigueperse et al., 1999). *In vivo*, ACTH suppression with dexamethasone treatment resulted in a marked decrease of *Akr1b7* mRNA levels that were restored when the treated mice were injected with exogenous ACTH. This ACTH or cAMP-induced Akr1b7 transcription was blocked by a PKA inhibitor (H89) in the murine adrenocortical ATC and Y1 cell lines, respectively (Aigueperse et al., 1999; Ragazzon et al., 2006).

Adrenal expression and ACTH regulation of Akr1b7 are supported by three SF-1 binding sites and other *cis*-elements located in the 5′-flanking regulatory region of the gene. Using transgenic mice and cell transfection experiments, we delimited a cryptic SF-1 response element (SFRE) 102 bp upstream of the transcription start site. This SFRE supported basal adrenal promoter activity. Two other SFREs were identified further upstream.

Table 2 | Localization and regulation of AKR1B expression in adrenal gland.

Isoforms	Localization	Analyses	Control by ACTH/ cAMP	Transcriptional regulators	Reference
HUMAN					
AKR1B1	Cortex	IHC, RNA Master blot	+	n.d.	a,b
AKR1B10	Adrenal*	RNA master blot	n.d.	n.d.	b
AKR1B15	n.d.	n.d.	n.d.	n.d.	–
MOUSE					
Akr1b3	Cortex + medulla	WB	No	No	a,c
Akr1b7	Cortex	NB, WB, IHC, ISH	+	Sp1, C/EBPβ, SF-1	a,c,d,e,f,g
Akr1b8	Cortex	WB, ISH	No	No	a,c,d
Akr1b16	n.d.	n.d.	n.d.	n.d.	–
RAT					
Akr1b4	Cortex	RT-PCR, IHC, WB	n.d.	n.d.	h,i
Akr1b13	Adrenal*	RT-PCR	n.d.	n.d.	h
Akr1b14	Cortex	ISH, WB	+	n.d.	j

*, *Intra-adrenal tissular localization was not specified; n.d., not determined; NB, Northern blot; WB, Western blot; RT-PCR, reverse transcription-polymerase chain reaction; ISH, in situ hybridization; IHC, immunohistochemistry.*

[a]*Lambert-Langlais et al. (2009)*, [b]*Hyndman and Flynn (1998)*, [c]*Martinez et al. (2001)*, [d]*Lau et al. (1995)*, [e]*Aigueperse et al. (1999)*, [f]*Val et al. (2004)*, [g]*Aigueperse et al. (2001)*, [h]*Endo et al. (2009a)*, [i]*MacLeod et al. (2010)*.

Table 3 | Kinetic constants of AKR1B toward 4-hydroxynonenal, isocaproaldehyde, and prostaglandin H2.

Substrates	4-Hydroxynonenal		Isocaproaldehyde		Prostaglandin H2[e]	
	K_m (μM)	K_{cat} (s^{-1})	K_m (μM)	K_{cat} (s^{-1})	K_m (μM)	V_{max} (nmol/min/mg)
HUMAN						
AKR1B1	716[a]	0.84[a]	1[b]	0.66[b]	1.9	44
AKR1B10	31[a]	2.01[a]	330[c]	0.72[c]		No activity
AKR1B15	n.d.	n.d.	n.d.	n.d.		n.d.
MOUSE						
Akr1b3	665[a]	0.82[a]	62[d]	1.3[d]	9.3	26
Akr1b7	256[a]	0.1[a]	320[d]	0.38[d]	3.8	53.4
Akr1b8	230[a]	3.18[a]	71[d]	0.03[d]		No activity
Akr1b16	n.d.	n.d.	n.d.	n.d.		n.d.
RAT						
Akr1b4	33[f]	0.23[f]	n.d.	n.d.		n.d.
r-Akr1b10	1.6[g]	0.05[g]	11[g]	0.03[g]		n.d.
Akr1b13	30[f]	0.18[f]	n.d.	n.d.		n.d.
Akr1b14	7.6[h]	0.02[h]	16[h]	0.03[h]		n.d.

n.d., not determined.

References (for comparison data were converted in μM for the K_m and in s^{-1} for the K_{cat}): [a]*Joshi et al. (2010)*, [b]*Matsuura et al. (1996)*, [c]*Martin and Maser (2009)*, [d]*Martinez et al. (2001)*, [e]*Kabututu et al. (2009)*, [f]*Endo et al. (2009a)*, [g]*Endo et al. (2010b)*, [h]*Endo et al. (2010a)*.

The site at −458 was a *bona fide* SFRE playing an essential role for both basal promoter activity and cAMP responsiveness whereas the site at −503 conferred intrinsic cAMP-sensing ability (Martinez et al., 2003; Val et al., 2004). Two other binding sites for the *trans*-acting factors Sp1 and C/EBPβ at position −52 and −61, respectively, also contributed to the transcriptional cAMP responsiveness (Aigueperse et al., 2001; **Table 2** and **Figure 1**).

Aldose reductases are able to reduce isocaproaldehyde that is produced in large amount in the adrenal cortex during steroidogenesis. Indeed, the first step of steroidogenesis is the removal of the cholesterol side chain by the P450scc enzyme, resulting in the formation of pregnenolone and isocaproaldehyde. Furthermore, isocaproaldehyde is a cytotoxic aldehyde whose accumulation in Y1 cells decreased their viability (Lefrancois-Martinez et al., 1999). *In vitro* studies revealed that Akr1b3, Akr1b7, and Akr1b8 all had

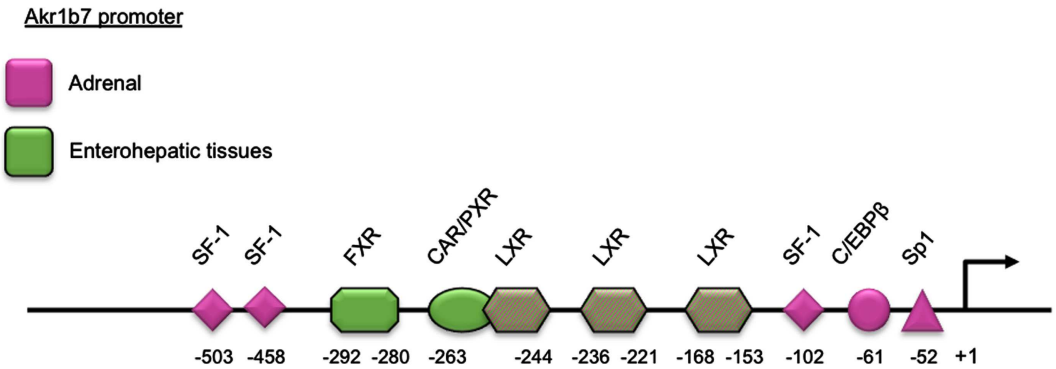

FIGURE 1 | Schematic representation of the *Akr1b7* promoter. The DNA binding site for transcription factors and nuclear receptors required for the *Akr1b7* specific expression in adrenal gland (pink boxes) and in enterohepatic tissue (green boxes) are shown. LXR binding sites are involved in both adrenal and intestine *Akr1b7* expression.

the ability to reduce isocaproaldehyde. Their kinetic constants suggested that isocaproaldehyde was a major substrate for Akr1b3 and Akr1b7, and a poor substrate for Akr1b8 (Martinez et al., 2001; **Table 3**). Akr1b7 silencing in Y1 adrenocortical cells disrupted cAMP-induced isocaproaldehyde reductase activity. This strongly suggested that Akr1b7, rather than Akr1b3, was the main isocaproaldehyde reductase in the adrenal gland (Lefrancois-Martinez et al., 1999). These observations further indicate that ACTH not only coordinates expression of enzymes responsible for the biosynthesis of steroids, but also of non-steroidogenic enzymes involved in the detoxification of reactive aldehydes generated during steroidogenesis.

In a previous study, Madore and colleagues proposed that AKR1B5, initially characterized as the bovine 20α-hydroxysteroid dehydrogenase, ensured prostaglandin F2α synthase (PGFS) activity in the endometrium (Madore et al., 2003). Thereafter, we have established by *in vitro* studies, that this property could be extended to other but not all AKR1B enzymes. Indeed, AKR1B1, Akr1b3, and Akr1b7 were shown to catalyze the reduction of prostaglandin H_2 (PGH_2) into $PGF_{2\alpha}$ (**Table 3**). In contrast, Akr1b8 and AKR1B10 recombinant proteins were devoid of this PGH_2 9-,11-endoperoxide reductase activity. This activity has not been investigated for Akr1b16, AKR1B15, and rat AR so far. Importantly, based on their kinetic parameters, recombinant AKR1B1, Akr1b3, and Akr1b7 displayed better PGF synthase activities than the previously characterized PGF synthases in mammals (Kabututu et al., 2009).

Prostaglandins are paracrine/autocrine cell mediators sharing a common precursor, PGH_2, which is synthesized from free arachidonic acid by the cyclooxygenases type 1 (COX-1) or type 2 (COX-2). COX-1 is regarded as a constitutively expressed enzyme. COX-2, on the other hand, is undetectable in most tissues in basal conditions but can be induced by various mitogenic agents and inflammatory stimuli (Ramsay et al., 2003). Following these observations, we carefully examined the $PGF_{2\alpha}$ biosynthetic pathway in the adrenal gland (Lambert-Langlais et al., 2009).

$PGF_{2\alpha}$ was secreted by both cortical (steroidogenic cells) and medullary (chromaffin cells) compartments of the adrenal gland. In primary adrenocortical cell culture, $PGF_{2\alpha}$ release was induced 2.5-fold by ACTH exposure which was correlated with ACTH-responsiveness of both COX-2 and Akr1b7. Using over- and down-expression strategies in cell lines, we demonstrated the pivotal role of Akr1b7 in ACTH-induced $PGF_{2\alpha}$ release and its functional coupling with COX-2. In the adrenal medulla, $PGF_{2\alpha}$ production seemed to be resulting from the coordinated activities of Akr1b3 and COX-1. In the adrenal, expression of the $PGF_{2\alpha}$ specific receptor (FP) was restricted to chromaffin cells, suggesting that both autocrine (within the medulla) and paracrine (between steroidogenic and chromaffin cells) mechanisms were relaying $PGF_{2\alpha}$ action. Indeed in the chromaffin cell line MPC862L, $PGF_{2\alpha}$ was able to repress both basal and glucocorticoid-induced dopamine release. By comparing $PGF_{2\alpha}$-responsiveness of isolated cells and whole adrenal cultures, we demonstrated that $PGF_{2\alpha}$ repressed glucocorticoid secretion by an indirect mechanism involving a decrease in catecholamine release, which in turn decreased adrenal steroidogenesis.

These results allowed us to propose a new mechanism for an intra-adrenal feedback loop, in which AR play a pivotal role in the regulation of adrenal endocrine functions (**Figure 2**). The mechanism that we proposed was the following: (1) In basal conditions, $PGF_{2\alpha}$ is constitutively secreted by chromaffin cells (by the coupling of COX-1 and Akr1b3), thus regulating catecholamines production and also limiting their paracrine action on steroidogenesis. (2) During a stress situation, ACTH transiently induces COX-2 and Akr1b7 expression, which results in $PGF_{2\alpha}$ production inside the cortex. $PGF_{2\alpha}$ produced in the cortex then represses catecholamines release by the medulla, via a paracrine action on its FP receptor. Decreased catecholamines release in turn reduces the effect of ACTH on glucocorticoids production (Lambert-Langlais et al., 2009).

Akr1b3/AKR1B1 EXPRESSION PROFILE, DETOXIFICATION FUNCTION, AND PARACRINE ACTION

By using different adrenal cell lines, we managed to detect Ak1b3 protein in the adrenocortical Y1 cells, and in the chromaffin MPC862L cells. These results confirmed that, unlike other murine AR, Akr1b3 is constitutively expressed in the whole adrenal gland (Lambert-Langlais et al., 2009). In Y1 adrenocortical cell line

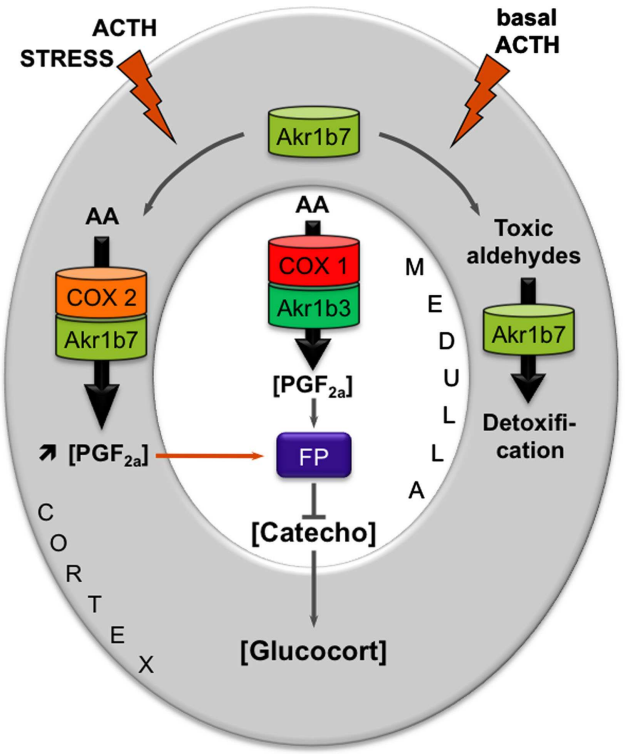

FIGURE 2 | Proposed model integrating dual functions of aldose reductases in the regulation of adrenal endocrine functions. Free arachidonic acid (AA) is metabolized into PGH_2 by COX enzymes and then converted into $PGF_{2\alpha}$ by PGFS of the AKR1B family. FP receptor expression is restricted to the medullary zone. $PGF_{2\alpha}$ synthesized in both the cortex and medulla thus signals in an autocrine/paracrine manner on chromaffin cells. This inhibits catecholamines production. Catecholamines produced in the medulla normally stimulate glucocorticoids release by the cortex. Decreased catecholamines production in response to $PGF_{2\alpha}$ stimulation thus results in a decrease in glucocorticoids production. The differential expression and regulation of both COX and AKR1B enzymes within the adrenal zones could allow the adjustment of $PGF_{2\alpha}$ production to limit stress response or control basal steroidogenesis by finely tuning glucocorticoids secretion. In basal conditions, chromaffin cells of the medullary zone constitutively secrete $PGF_{2\alpha}$, through the functional coupling between COX-1 and possibly the PGFS Akr1b3. Under stress conditions, the resulting ACTH surge induces COX-2 expression and sustains Akr1b7 levels in the cortex. The functional coupling between COX-2 and Akr1b7 triggers a $PGF_{2\alpha}$ surge that could act as a local paracrine feedback to limit catecholamine-mediated glucocorticoid release. After the stress response has ended, COX-2 returns to undetectable levels. The coupling between Akr1b7 and COX-2 does not take place. Akr1b7 then functions only as a detoxifying enzyme of the harmful aldehydes produced under chronic/basal stimulation of steroidogenesis. Catecho, catecholamines; Gluco, glucocorticoids.

and during *in vivo* hormonal manipulations, cAMP appeared to have no significant effect on Akr1b3 expression suggesting that its expression was insensitive to ACTH (Martinez et al., 2001; **Table 2**).

Akr1b3 is also involved in the detoxification of toxic carbonyls. Even if Akr1b7 is the main isocaproaldehyde reductase and Akr1b8 the principal 4-HNE reductase, Akr1b3 is also able to reduce these toxic compounds. This suggests that Akr1b3 can take part in the elimination of these compounds in basal physiological conditions (Lefrancois-Martinez et al., 1999; Martinez et al., 2001). Moreover, Akr1b3 is constitutively expressed in cortical and medullary cells, where it could be coupled to COX-1 to synthesize $PGF_{2\alpha}$ (see above paragraph).

Despite its expected participation in the elimination of toxic compounds and in the production of signal molecules ($PGF_{2\alpha}$), there is no report of adrenal dysfunction in mice lacking Akr1b3 (*Akr1b3*$^{-/-}$; Aida et al., 2000; Ho et al., 2000). This absence of adrenal phenotype may result from functional redundancy between the different family members present in the gland (Akr1b7 and Akr1b8).

AKR1B1 expression has initially been detected using a RNA master blot in human adrenal gland (Hyndman and Flynn, 1998). More recently, immunohistochemistry experiments allowed us to assign AKR1B1 to the cortical compartment of the gland (Lambert-Langlais et al., 2009). In NC1-H295, a human adrenocortical tumor cell line, *AKR1B1* mRNA levels were induced by forskolin (adenylyl cyclase inducer) treatment (Lefrançois-Martinez et al., 2004). This suggested that similarly to *Akr1b7*, *AKR1B1* gene expression could be sensitive to ACTH control. However, the molecular mechanisms and *cis*-acting elements ensuring ACTH/cAMP responsiveness of *AKR1B1* gene have remained unexplored.

For years, AKR1B1 had been considered as the major isocaproaldehyde reductase in the adrenal gland (Matsuura et al., 1996). The NADPH-dependent isocaproaldehyde reductase activity harbored by AKR1B1 was inhibited by tolrestat while the murine isocaproaldehyde reductase Akr1b7 was insensitive to this pharmacological inhibitor (Matsuura et al., 1996; Lefrancois-Martinez et al., 1999). We demonstrated that AKR1B1 was also endowed with 9-,11-endoperoxide reductase activity (Kabututu et al., 2009). The conversion of PGH_2 into $PGF_{2\alpha}$, catalyzed by AKR1B1, strictly dependent on the presence of NADPH, was inhibited by tolrestat whereas Akr1b7 PGFS activity was insensitive to AR inhibitors.

We showed that in the normal human adrenal gland, AKR1B1 and COX-2 were co-localized in steroidogenic cortical cells (Lambert-Langlais et al., 2009). Therefore, the human adrenal cortex could also have the potential to produce $PGF_{2\alpha}$ in response to ACTH. With respect to their hormonal regulation in the adrenal cortex and their reductase activity toward common substrates, we have postulated that AKR1B1 could be considered as a functional ortholog of Akr1b7 in the human adrenal cortex (Lefrançois-Martinez et al., 2004). The possibility that the PGFS activity of AKR1B1 could be involved in an intra-adrenal feedback loop between endocrine activities of cortical and medullary compartments in human adrenal gland remains to be explored.

Given the high expression of AKR1B1 in the adrenal cortex we evaluated alterations in its expression in association with human adrenal disorders. The relative abundance of *AKR1B1* mRNA was decreased in ACCs when compared to benign tumors, Cushing's hyperplasia, or normal adrenals (Lefrançois-Martinez et al., 2004). These data provided evidence that expression of AKR1B1 was decreased in adrenocortical cancer. This was further confirmed by the unsupervised clustering analysis of the human adrenal tumors transcriptome performed by de Reyniès et al. (2009), indicating

that decreased expression of AKR1B1 correlated with malignancy for the molecular diagnosis of adrenal tumors.

AKR1B AND GLUCIDO-LIPIDIC HOMEOSTASIS

AKR1B IN ENTEROHEPATIC TISSUES

Excessive nutrient intake is detrimental for cells and tissues. In mammals, the liver converts excess dietary carbohydrates into triglycerides through *de novo* lipogenesis. Two transcription factors, carbohydrate-responsive element-binding protein (ChREBP) and sterol responsive element-binding protein-1c (SREBP-1c) emerged as major mediators of glucose and insulin action in the control of both glycolysis and lipogenesis in the liver. The liver X receptors (LXR) are oxysterol activated transcription factors acting as important metabolic regulators of the lipogenic pathway. Indeed, LXRs ensure the transcriptional control of SREBP-1c in response to insulin and of ChREBP. Moreover, direct targets of LXRs include other lipogenic genes such as *fatty acid synthase* (*Fas*) and *stearoyl-CoA desaturase 1* (*SCD1*; Chen et al., 2004; Postic and Girard, 2008; Kim et al., 2009). The farnesoid X receptor (FXR)/bile acid receptor is another nuclear receptor that plays an important role in maintaining bile acid, lipid, and glucose homeostasis since its activation has been shown to lower blood triglyceride and cholesterol levels and to improve insulin sensitivity in diabetic mouse models (Zhang and Edwards, 2008). In enterocytes, FXR was shown to protect against the cytotoxic effects of bile acids by increasing expression of binding proteins and transporters (Schmidt and Mangelsdorf, 2008).

Akr1b8/AKR1B10: from cell detoxification to lipid synthesis

Previous analyses performed by RNAse protection assays reported very weak levels of *Akr1b8* transcript (Lau et al., 1995) in the mouse intestine and absence of expression in the adult liver. These observations were partially revised by Joshi et al. (2010) who showed high *Akr1b8* expression all along the adult intestinal tract and moderate expression in the liver. *Akr1b8* expression was initially described to be up-regulated during the early phase of the cell cycle and induced by growth promoting agents [fibroblast growth factor (FGF) and epidermal growth factor (EGF)], as well as by hypertonic stress (Donohue et al., 1994; Hsu et al., 1997). Although *Akr1b8* displayed a strong expression in mouse colon cancer cells (Joshi et al., 2010) and was suggested to be a target gene for NF-E2 related factor 2 (Nrf2) transcription factor that controls intestinal detoxification response (Varady et al., 2011), its expression and regulation in healthy liver and intestine remained elusive. A recent study investigating bile acids impacts on *Akr1b7* expression showed that enterohepatic expression of *Akr1b8* was insensitive to bile acids (Schmidt et al., 2011). Akr1b8 enzymatic activities were extensively studied regarding its ability to reduce the highly reactive 4-HNE produced during peroxidation of polyunsaturated fatty acids (Srivastava et al., 1998). *In vitro* enzymatic studies reported that multiple members of the AKR1B group, including Akr1b8, were endowed with the ability to reduce phospholipid aldehydes derived from lipid peroxidation. Compared with AKR1B1 or Akr1b7, Akr1b8 activity was more efficient with the reduction of short chain aldehydes such as 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine and 1-pamitoyl-2-arachidonoyl-*sn*-glycero-3-phosphoglycerol (Spite et al., 2007). In line with the high lipid metabolic activities in Akr1b8 producing sites, it was proposed that Akr1b8 was acting physiologically as a scavenger of toxic aldehydes derived from peroxidation of endogenous or dietary lipids.

Beside its aldehyde reductase activity, Akr1b8 was shown to associate with the lipogenic acetyl-CoA carboxylase α (ACCA) in murine colon cancer cells (Joshi et al., 2010). ACCA is a rate-limiting enzyme of *de novo* synthesis of fatty acids, catalyzing the formation of malonyl-coA by ATP-dependent carboxylation of acetyl-coA. This interaction protects ACCA from proteasomal degradation and consequently allows increased fatty acid synthesis that leads to production of lipid second messengers that promote cell proliferation (Chajès et al., 2006). Whether Akr1b8 is also involved in the stability of ACCA in healthy enterocytes has not yet been determined. In order to associate gene signature with metabolic network, a genetic study integrating quantitative trait locus (QTL) mapping and network modeling led to knock-out (KO) the three corresponding identified genes, among which *Akr1b8* (Derry et al., 2010). *Akr1b8*$^{-/-}$ mice phenotype was very briefly described in this study suggesting a more specific perturbation in fat tissue homeostasis rather than in the liver or intestine (see below).

The *AKR1B10* gene has recently been determined as the human ortholog of *Akr1b8* (Joshi et al., 2010). Its expression was primarily detected in healthy colon, small intestine, and liver (Cao et al., 1998) and was found up-regulated with their corresponding tumorigenic transformation (Cao et al., 1998; Yan et al., 2007; Heringlake et al., 2010; Liu et al., 2012). This was also observed in lung and breast cancers suggesting that AKR1B10 overexpression could be associated with a broader tumor phenotype. In human hepatocarcinoma, insulin or EGF enhanced *AKR1B10* expression through the activator protein-1 (AP1) mitogenic signaling. This enhanced tumor development and progression through elimination of cytotoxic carbonyls and promotion of lipogenesis (Liu et al., 2012). The treatment of human colon cancer SW-480 and HT-29 cell lines with proteasome inhibitors known to increase the expression of Nrf2-regulated genes induced AKR1B10 expression suggesting that AKR1B10 could be a target of the Nrf2 transcription factor (Ebert et al., 2011). Nrf2 responsiveness of *AKR1B10* gene was further demonstrated by co-transfection experiments of *AKR1B10* promoter luciferase reporter constructs in human lung adenocarcinoma cell lines (Nishinaka et al., 2011; **Figure 3**).

AKR1B10 was the first AKR1B protein which was demonstrated to enhance cell proliferation and promote cell survival through its ability to interact with and stabilize ACCA in breast cancer cells (Ma et al., 2008) and in colon cancer cells (Wang et al., 2009). AKR1B10 mediates ACCA stability through physical association. This was shown to affect fatty acid/lipid synthesis, mitochondrial function, and oxidative status. Identification of Akr1b8/AKR1B10 protein domain interacting with ACCA would be helpful to predict this characteristic for other AKR1B proteins and to develop targeted therapeutic strategies for the modulation of *de novo* fatty acid synthesis in cancer cells.

We are exposed daily to α,β-unsaturated aldehyde and *trans*-2-hexenal through food and drink consumption (Stout et al., 2008). This persistent exposure to electrophilic carbonyls requires an effective defense system to protect intestinal cells from irreversible damage. Similarly to Akr1b8, *in vitro* enzymatic studies on

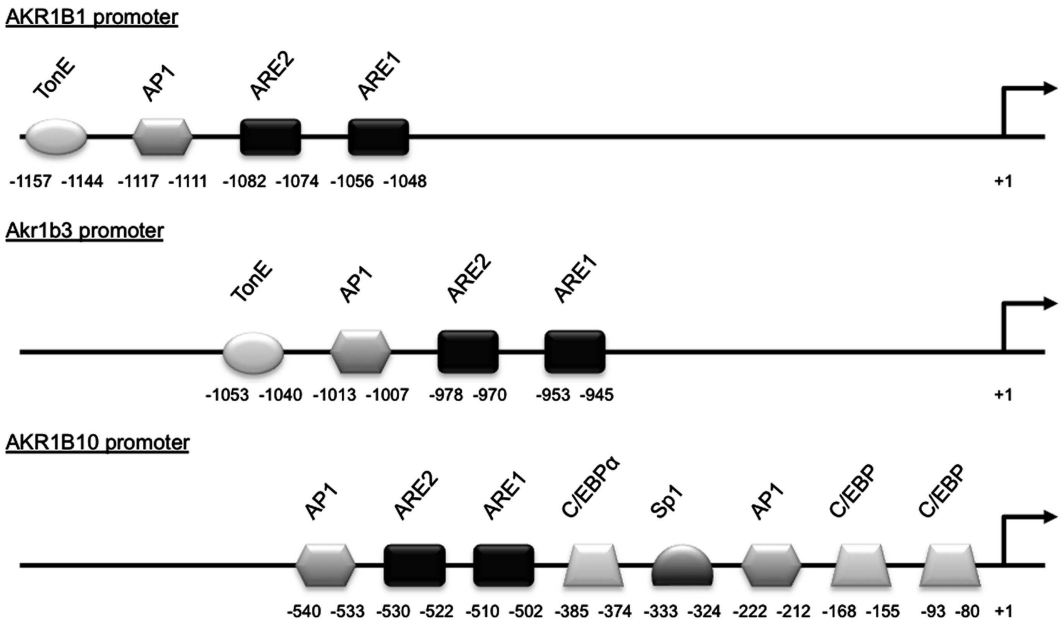

FIGURE 3 | Schematic representation of the *AKR1B1, Akr1b3,* and *AKR1B10* promoters. *Cis*- and *trans*-acting factors shown to be involved in stress responsiveness are indicated. TonE, tonicity response element; AP1, activator protein-1 binding site; ARE, antioxidant response element; C/EBP, CCAAT enhancer binding protein binding site; Sp1, selective promoter factor 1 binding site.

AKR1B10 demonstrated a highly specific ability to reduce physiological levels of 4-HNE and other alpha and beta-unsaturated carbonyls in both their free or glutathione-conjugated form (Martin and Maser, 2009; Zhong et al., 2009). Expression of AKR1B10 may reflect the requirement for intestinal cells to protect against the dietary, lumen microbial and lipid-derived carbonyls.

Although physiological integration of all these data remains difficult, this suggests that the beneficial role of Akr1b8/AKR1B10 in the protection of healthy cells against lipid peroxidation could switch, in the case of cancer, into a deleterious role promoting cell proliferation through stabilization of ACCA (**Figure 4**).

Akr1b7: improving (liver) metabolic capacity

A target for metabolic nuclear receptors (Figure 1 and Table 4).

Several studies reported specific *Akr1b7* expression in the small intestine (Volle et al., 2004; Ge et al., 2011; Schmidt et al., 2011) within the epithelial cells of the villi (Lau et al., 1995) with a decreasing gradient from the duodenum to the ileum. This expression has been shown to be under the control of oxysterol activated LXR/retinoic X receptor (RXR) heterodimers acting through three LXR response elements (LXRE) located in the *Akr1b7* proximal promoter (LXRE3: −259 to −244, LXRE1: −236 to −221, LXRE2: −153 to −168 from the transcription start site). Two of these *cis*-acting elements were specific for regulation by the alpha LXR isoform (Volle et al., 2004).

Two other recent reports showed that *Akr1b7* gene transcription in the small intestine, colon and liver was also controlled by bile acids through a FXR response elements (FXRE) located at position −292 to −280 on the promoter (Ge et al., 2011; Schmidt et al., 2011).

Several nuclear receptors, including FXR, pregnane X receptor (PXR) and constitutive androstane receptor (CAR), have been shown to protect against the cytotoxic effects of bile acids by increasing expression of binding proteins, transporters, and enzymes that detoxify bile acids (Zollner et al., 2006; Schmidt and Mangelsdorf, 2008). The combined use of KO mouse models for PXR/CAR receptors and pharmacological activators allowed identification of *Akr1b7* as one of their target genes in the liver (Liu et al., 2009; **Table 4** and **Figure 1**).

Although molecular mechanisms mediating metabolic regulation of *Akr1b7* expression in the liver and intestine were profusely examined using genetic models or forced expression systems, there is no direct evidence for Akr1b7 expression in liver sections or in isolated hepatocytes, to date.

Detoxifying lipid aldehydes. Wild-type mice treated with PCN (pregnenolone-16α-carbonitrile; a PXR agonist) exhibited a decrease in malondialdehyde (MDA) levels (another by-product of polyunsaturated fatty acid peroxidation) compared with their vehicle-treated counterparts. The effect of PCN on MDA was abolished in $Pxr^{-/-}$ mice. The authors suggested that PXR played a role in alleviating lipid peroxidation in small intestine. Since the induction of *Akr1b7* expression by PCN was also abolished by PXR KO, the effects of PXR on lipid peroxidation could be mediated, at least in part, by modulation of *Akr1b7* expression (Liu et al., 2009; Ge et al., 2011; Schmidt et al., 2011).

In light of the ability of Akr1b7 to reduce 4-HNE, Volle et al. have compared the status of lipid peroxidation in the small intestine of wild-type and $Lxr\alpha^{-/-}$ mice by measuring MDA levels. In the duodenum, a significant decrease in MDA concentrations was seen when wild-type mice were treated with T091317 (a synthetic

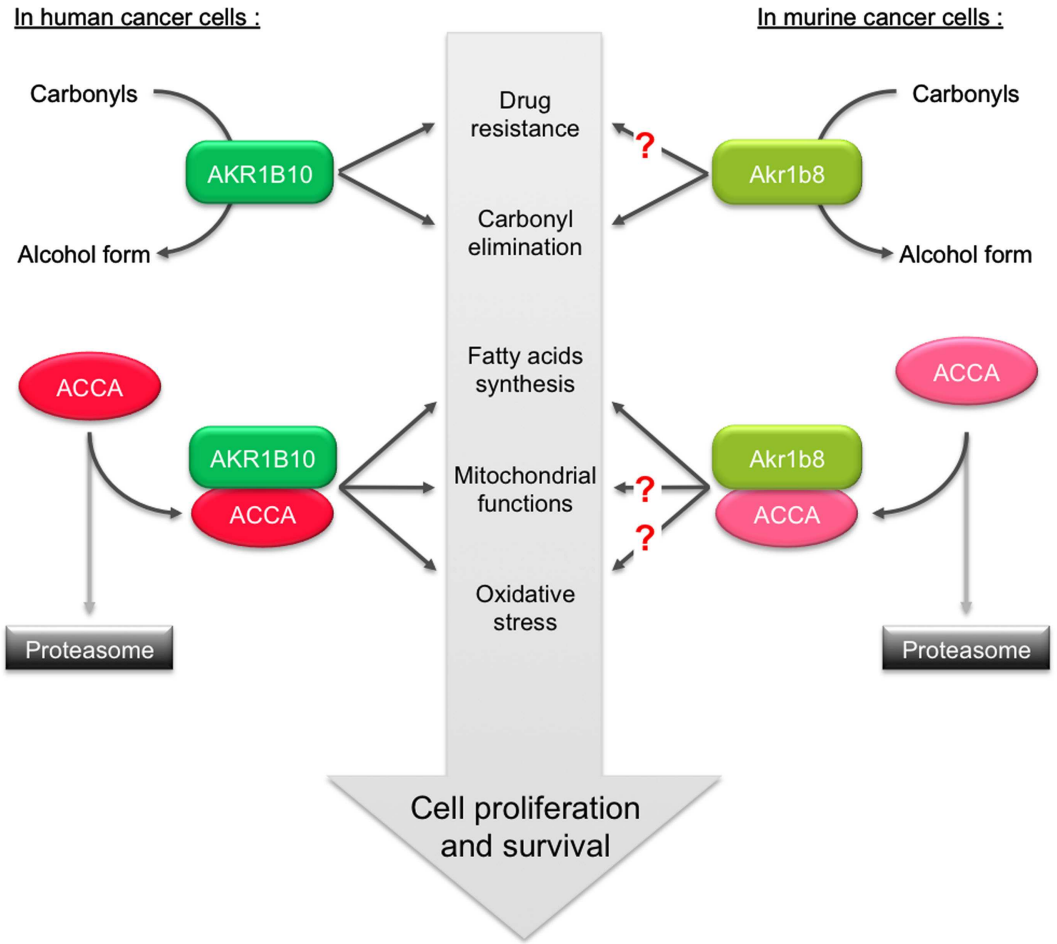

FIGURE 4 | Role of AKR1B10 and Akr1b8 in the regulation of lipid synthesis and contribution in promoting cancer. In human cancer cells, ACCA is protected against ubiquitination-proteasome dependent degradation through its association with AKR1B10. Stabilization of ACCA increases fatty acids synthesis. This protects cell from apoptosis by preventing depletion of phospholipids, mitochondrial membrane lesions, and oxidative stress. The dual capacity of AKR1B10 to stimulate lipogenesis and to detoxify carbonyls and drugs thus contributes to enhance cell proliferation/survival. In murine cancer cells, Akr1b8 was also shown to stabilize ACCA thereby modulating fatty acid synthesis. The impact of Akr1b8-mediated ACCA stabilization on mitochondrial function and oxidative stress is still unknown.

agonist of LXR). This effect was not observed in *Lxr*$\alpha^{-/-}$ mice, where T091317 was unable to induce *Akr1b7*. The authors suggested that increased levels of LXRα in the small intestine and its activation by oxysterols could in turn, up-regulate the expression of detoxifying genes which could ultimately reduce oxidative stress (Volle et al., 2004). However, although the decrease in MDA contents is clearly a LXR dependent mechanism, the direct involvement of Akr1b7 seems unlikely. Indeed overexpression of Akr1b7 *in vivo* does not alter MDA levels (Ge et al., 2011).

Detoxifying bile acids or lipid peroxidation induced by bile acids excess (Figure 5). Recently three independent studies proposed that *Akr1b7* or its rat ortholog *Akr1b14* could be involved in bile acid metabolism and/or signaling (Endo et al., 2011; Ge et al., 2011; Schmidt et al., 2011).

Bile acids are cholesterol-derived molecules produced for its solubilization in the gallbladder and intestine by forming mixed micelles with cholesterol and phospholipids. They are required for the activation of some pancreatic enzymes and for the absorption of cholesterol, lipid soluble vitamins and to a lesser extent, triglycerides and fatty acids from the intestine (Hylemon et al., 2009). They also act as signaling molecules by acting as ligands for several nuclear receptors including FXR and PXR or the membrane $G\alpha_s$ protein-coupled receptors TGR5 as well as $G\alpha_i$ protein-coupled receptors. Hence, bile acids contribute to the regulation of their own synthesis, fatty acid, lipid, and lipoprotein synthesis as well as glucose metabolism in the liver.

An *in vitro* enzymatic study using recombinant Akr1b14 (rat) and Akr1b7 (mouse) proteins showed that conjugated and unconjugated bile acids (chenodeoxycholic acid (CDCA)/glyco-CDCA and hyodeoxycolic acid (HDCA)/glyco-HDCA) specifically, quickly, and significantly activated the NADPH-linked reductase activity of Akr1b14 and to a lesser extent Akr1b7 activity (Endo et al., 2011). More accurately, higher specific activation of Akr1b14 was mediated by an interaction of bile acids with the His269 facilitating the release of $NADP^+$. In Akr1b7, the histidine

Table 4 | Expression of aldose reductases in liver and small intestine.

Isoforms	Liver	Small intestine	Analyses	Tissue-specific transcriptional regulators	Reference
HUMAN					
AKR1B1	+	+	NB, WB	Nrf2	a,b,c
AKR1B10	+	+	NB, WB, IHC	AP1, Nrf2	a,c,d,e,f,g,h
AKR1B15	n.d.	n.d.	n.d.	n.d.	–
MOUSE					
Akr1b3	+	+	RT-PCR	Nrf2	i,k
Akr1b7	+	+	RNase protection, RT-qPCR, WB	LXR, CAR, PXR, FXR	j,l,m,n,o,p,q
Akr1b8	+	+	RT-PCR, NB	Nrf2	i,r,s,t
Akr1b16	+	n.d.	RT-PCR	n.d.	s
RAT					
Akr1b4	+	+	RT-PCR, WB	n.d.	u,v
r-Akr1b10	+	–	RT-PCR	n.d.	w
Akr1b13	+	+	RT-PCR, WB	n.d.	u,w
Akr1b14	+	–	RT-PCR	n.d.	u,w

n.d., not determined; NB, Northern blot; WB, Western blot; RT-PCR, reverse transcription-polymerase chain reaction.

[a] Cao et al. (1998), [b] O'Connor et al. (1999), [c] Ebert et al. (2011), [d] Fukumoto et al. (2005), [e] Martin et al. (2006), [f] Heringlake et al. (2010), [g] Liu et al. (2012), [h] Nishinaka et al. (2011), [i] Joshi et al. (2010), [j] Lau et al. (1995), [k] Nishinaka and Yabe-Nishimura (2005), [l] Volle et al. (2004), [m] Schmidt et al. (2011), [n] Ge et al. (2011), [o] Aigueperse et al. (1999), [p] Lambert-Langlais et al. (2009), [q] Martinez et al. (2001), [r] Donohue et al. (1994), [s] Salabei et al. (2011), [t] Rangasamy et al. (2004), [u] Endo et al. (2009b), [v] MacLeod et al. (2010), [w] Endo et al. (2010b).

residue found at position 269 in the Akr1b14 protein is replaced by an arginine residue. This may account for weaker activation by bile acids. The authors suggested that bile acids could activate Akr1b14/Akr1b7 when their blood concentrations were elevated. As high concentrations of hydrophobic bile acids could induce cell injury through several pathways, e.g., lipid peroxidation, activation of Akr1b14 by bile acids may increase detoxification of the harmful lipid peroxidation by-products, and contribute to the attenuation of toxic effects of bile acids.

Because members of the AKR family (i.e., AKR1C4 and AKR1D1) were shown to be involved in the bile acid synthesis, Mangelsdorf's group hypothesized that FXR-inducible Akr1b7 could be involved in bile acid metabolism in the intestine (Schmidt et al., 2011). They showed that Akr1b7 overexpression in heterologous HEK293 cells was associated with a reduction activity of 3-keto bile acids to their 3β-hydroxy derivatives, which are less toxic than their 3α-hydroxy epimers. Altogether, these results suggest a novel function for Akr1b7 in the detoxification of bile acids.

Possible role in metabolic action of bile acids signaling (Figure 5). In the liver, bile acids are able to influence glucose metabolism by at least two mechanisms. First, conjugated bile acids rapidly activate the insulin signaling pathway via $G\alpha_i$ protein-coupled receptors or superoxide ions. In this aspect, they function much like insulin through the AKT pathway to activate glycogen synthase and repress neoglucogenic genes (Hylemon et al., 2009). Importantly, bile acids also mediate another control on liver metabolism through the transcriptional activation of the gene encoding the small heterodimer partner (SHP) via a functional FXRE in its promoter. The orphan receptor SHP can exert inhibitory interactions with forkhead box O1 (FOXO1), C/EBPα, and hepatocyte nuclear factor 4α transcription factors, known to activate neoglucogenic genes (Yamagata et al., 2004). The inhibitory control of bile acids on gluconeogenesis has also been demonstrated in FXR null mice or with the treatment of diabetic animals with the FXR agonist GW4064 (Ma et al., 2006; Zhang et al., 2006). SHP is also able to interact with LXR to down-regulate the gene encoding SREBP-1c which is the predominant transactivator for genes encoding enzymes involved in fatty acid, triglyceride, and VLDL biosynthesis (Horton et al., 2002).

These observations led Ge et al. (2011) to investigate the implication of FXR target gene *Akr1b7* in liver glucose and lipid metabolism using adenovirus-mediated Akr1b7 overexpression. Overexpression of hepatic Akr1b7 significantly reduced plasma glucose levels. This was associated with reduced hepatic mRNA levels of gluconeogenic genes encoding phosphoenolpyruvate carboxykinase and glucose 6-phosphatase and of peroxisome proliferator-activated receptor γ co-activator 1α, as well as markedly increased hepatic SHP mRNA levels. Hepatic lipogenesis was also affected: mRNA levels of SREBP-1c and several lipogenic genes including Fas, diacylglycerol acyltransferase 2, cholesterol 7α-hydroxylase, and the ATP binding cassette G5 were significantly reduced. Nevertheless other FXR target genes such as the bile salt export protein or the multi drug resistance 2 were not affected. This excluded the hypothesis that FXR was activated through overexpression of Akr1b7. Forced hepatic expression of Akr1b7 also significantly lowered plasma glucose and hepatic triglyceride and cholesterol levels in db/db mice. These results show that overexpression of Akr1b7 in the liver selectively down-regulates gluconeogenic and lipogenic gene expression, even though the molecular mechanisms linking Akr1b7 to these metabolic changes are not elucidated. Yet, we can speculate that up-regulation of the

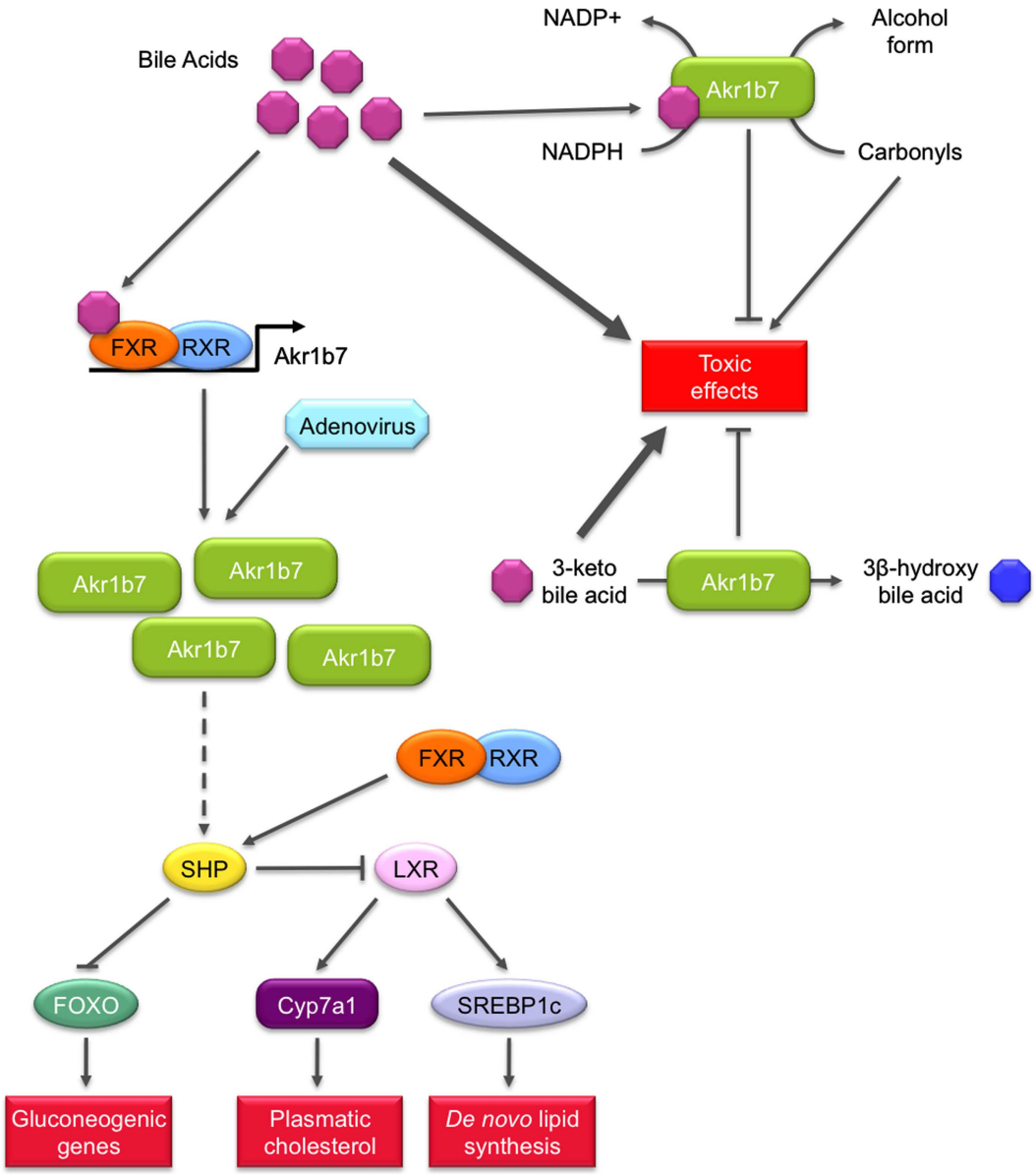

FIGURE 5 | Role of Akr1b7 in bile acids metabolism and signaling pathway. Akr1b7 has the ability to reduce 3-keto bile acids to their less toxic 3β-hydroxy derivatives. In turn, bile acids can stimulate detoxification activity of Akr1b7, enhancing its capacity to reduce carbonyl and 3-keto bile acids. In addition to bile acids detoxification, Akr1b7 is also one of their target genes. In liver and intestine, bile acids induce the FXR-dependent *Akr1b7* transcription. The forced expression of Akr1b7 in mouse liver by the mean of recombinant adenovirus results in the down-regulation of gluconeogenesis and lipid metabolism. Mechanisms involved are not unravel yet but could rely on the up-regulation of SHP expression. Increased SHP levels could in turn repress both FOXO and LXR, leading to the inhibition of gluconeogenic genes and lipogenic genes, respectively. The mechanism by which Akr1b7 accumulation modulates the SHP gene expression appears independent from FXR and remains to be discovered (dashed lines).

receptor SHP upon Akr1b7 overexpression has a central role in the coordinated repression of both gluconeogenic and lipogenic metabolisms.

Unlike overexpression of Akr1b8/AKR1B10 that leads to increased lipogenesis, overexpression of Akr1b7 in the liver reduced hepatic lipid accumulation suggesting that *in vivo* these closely related AKR1B proteins display non-overlapping functions. The possibility that other AR (including AKR1B10) endowed with 4-HNE reductase activity could modulate bile acid metabolism under the control of FXR, LXR, or PXR, remains to be determined. Transcripts for a novel murine ARLP (Akr1b16) were recently described in the liver but expression of Akr1b16 has not been investigated in the small intestine (Salabei et al., 2011). Moreover, mechanisms controlling their expression in these tissues have not yet been elucidated.

Akr1b3/AKR1B1: altering (liver) metabolic capacity

Both Akr1b3 and AKR1B1 are ubiquitously expressed. *Akr1b3* transcripts were detected in the liver and small intestine at similar levels (Joshi et al., 2010). *AKR1B1* messenger RNAs are found in the liver and small intestine (Cao et al., 1998). Western blot analyses confirmed AKR1B1 expression in the small intestine although it was undetectable in the normal adult liver. However AKR1B1 expression could be induced by alcoholic cirrhosis (O'Connor et al., 1999).

In diabetic mice, Akr1b3 is involved in glucose metabolism in some tissues through its participation in the polyol pathway. In response to chronic hyperglycemia, Akr1b3 catalyzes the rate-limiting reduction of glucose into sorbitol which is in turn converted into fructose by the sorbitol dehydrogenase (SDH). Activation of this pathway is involved in the development and progression of diabetic complications (Brownlee, 2001). Some evidences suggest that it could also be implicated in lipid metabolism. First, diabetic patients exhibit elevated blood triglycerides and non-esterified fatty acids and second, fructose-fed rats display an elevation of blood triglycerides and a reduction of peroxisome proliferator-activated receptor alpha (PPARα) activation (Roglans et al., 2007). These data led Qiu et al. to investigate the effects of hepatic activation of the Akr1b3/polyol pathway on lipid metabolism (**Figure 6**). In a murine hepatocyte cell line, overexpression of Akr1b3 was able to suppress PPARα transcriptional activity through extracellular signal-regulated kinase (ERK1/2)-mediated inactivating phosphorylations on Ser-12 and Ser-21. This resulted in decreased expression of genes involved in peroxisomal and mitochondrial β-oxidation pathway. In addition, Akr1b3 is up-regulated under high glucose concentrations in mouse hepatocyte AML12 cells and *in vivo* in diabetic mice. The genetic ablation or pharmacological inhibition of Akr1b3 in diabetic mice decreased ERK1/2-dependent phosphorylation of PPARα and blood triglycerides levels. In diabetic animals, SDH KO was accompanied by a reduction of blood triglycerides. This study clearly indicated that Akr1b3 and the polyol pathway sensed intracellular levels of glucose and adjusted PPARα activity through its phosphorylation/dephosphorylation, which in turn affected cellular lipid homeostasis (Qiu et al., 2008). In db/db mice, inhibition and knock-down of Akr1b3 induced the decrease of both plasma and liver triglycerides levels. Inhibition of Akr1b3 in db/db mice led to an improvement of hepatosteatosis due to the up-regulation of acetyl-coA oxidase and apolipoprotein A-V, two PPARα target genes involved in lipid catabolism (Qiu et al., 2012). Mechanisms by which Akr1b3 influences ERK-dependent inactivation of PPARα remain to be established.

AKR1B1 is also involved in the polyol pathway. Therefore, the relevance of these results in human remains to be determined. However, studies using *Akr1b3*$^{-/-}$ mice or transgenic mice over-expressing AKR1B1 revealed no abnormalities in either the liver or small intestine (Yamaoka et al., 1995; Aida et al., 2000).

In addition to this possible implication in metabolism, AKR1B1 could be an actor of small intestine protection against electrophilic carbonyls. Its expression is also induced in human colon cancer

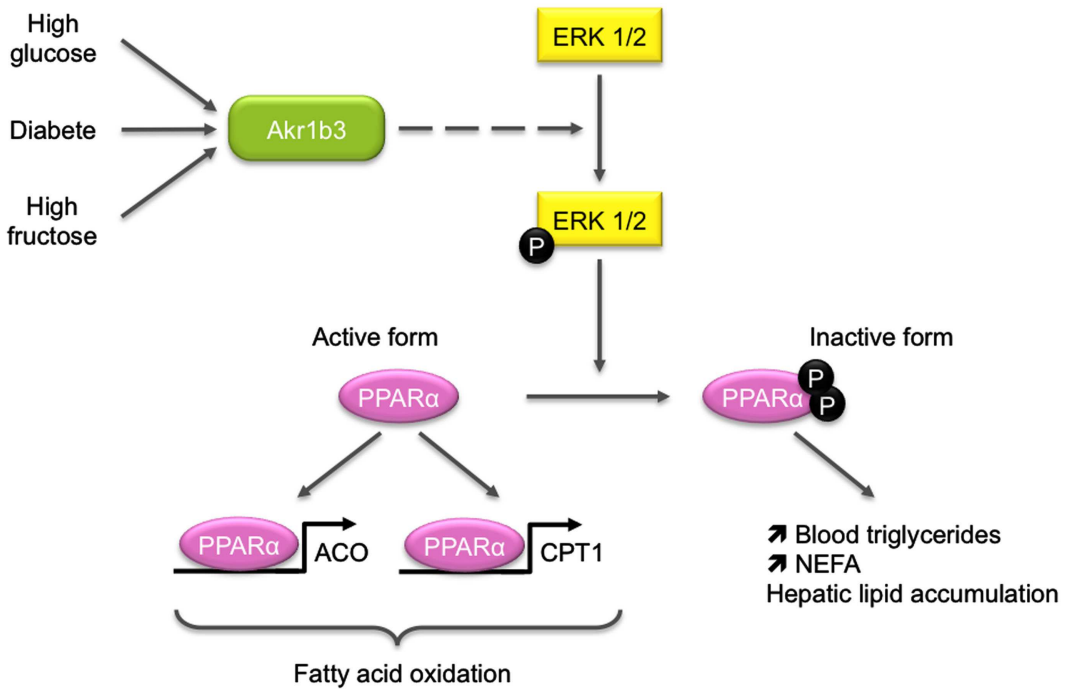

FIGURE 6 | Role of Akr1b3 in hepatic lipid homeostasis. Diabetic conditions (high glucose or high fructose) lead to *Akr1b3* up-regulation and Akr1b3-dependent ERK1/2 activation and are often associated to dyslipidemia. The ERK1/2 mediated phosphorylation of PPARγ inhibits its transcriptional activity resulting in down-regulation of β-oxidation master genes. Impaired β-oxidation favors hepatic lipid accumulation and increases blood triglycerides and non-esterified fatty acids (NEFA). The mechanism by which Akr1b3 induces ERK1/2 phosphorylation is still unknown (dashed lines).

cell lines SW-480 and HT-29, by proteasome inhibitors known to induce Nrf2 expression (Ebert et al., 2011). Characterization of *Akr1b3* promoter has shown that Nrf2 could regulate its activity through an antioxidant response element 1 (−953 to −945) and an AP1 site (Nishinaka and Yabe-Nishimura, 2005). The arrangement of these elements in the stress response region was conserved between *Akr1b3* and *AKR1B1* promoters (**Figure 3**).

Moreover, in a similar way as AKR1B10, AKR1B1 displayed enzymatic properties allowing protection of intestinal cells against dietary electrophilic carbonyls. Indeed, AKR1B1 kinetic parameters are compatible with reduction of glutathione-conjugated carbonyl compounds (Shen et al., 2011). Studies in healthy tissues could allow a better understanding of AKR1B1 implication in cytoprotection against oxidative damage.

AKR1B IN WHITE ADIPOSE TISSUE

For decades, adipose tissue was considered an inert mass of energy storage in which adipocytes were unique in the quantity of lipid that they can store during period of energy excess and mobilize as free fatty acids when required. In fat pads, adipocytes are intermingled with other cells including blood cells, endothelial cells, adipose precursors of varying degree of differentiation, and fibroblasts (Ailhaud et al., 1992). Adipose tissue plays a crucial role in the regulation of energy homeostasis, insulin sensitivity, and lipid/carbohydrate metabolism. These actions are mediated by both non-secreted proteins and hormones produced by adipocytes. These adipokines have wide-ranging effect on energy intake, energy expenditure, carbohydrate and lipid metabolism, including nutrient partitioning, and fuel selection (Trayhurn and Beattie, 2001; Fonseca-Alaniz et al., 2007; Vázquez-Vela et al., 2008). White adipose tissue expansion takes place rapidly after birth and it retains some plasticity throughout life (Frühbeck, 2008). Adipose precursors are able to differentiate in mature adipocytes and acquire features of fully differentiated cells during adipogenesis. Acquisition of the adipocyte phenotype is characterized by chronological changes in the expression of numerous genes. Studies on 3T3-L1 cells, a line of murine preadipocytes that have the ability to accumulate lipids during their hormonal-induced differentiation into adipocytes, have allowed identification of C/EBPs and PPARγ as master regulators of adipogenesis (Tontonoz et al., 1994; Wang et al., 1995; Tanaka et al., 1997; Rosen et al., 1999). The earliest event of adipogenesis is the transient induction of C/EBPβ and C/EBPδ expression, which in turn induces PPARγ and C/EBPα expression (Tang et al., 2004). This is followed by the synergic action of PPARγ and C/EBPα that enhance the expression of several genes characterizing the adipocyte phenotype along with massive triglyceride accumulation (Wu et al., 1999). SCD1, phosphoenolpyruvate carboxykinase, Fas, ACCA, malic enzyme, insulin receptor, adipocyte specific fatty acid binding protein, leptin (Rosen and Spiegelman, 2000) are some of the essential genes induced during terminal differentiation of adipocytes. Adipogenesis is tightly regulated by a set of pro- and anti-adipogenic factors including insulin, FGF, EGF, and prostaglandins (Gregoire et al., 1998). Adipose tissue expansion is resulting from adipocyte hyperplasia (generated by an increased number of adipocytes) and/or hypertrophy (caused by an enlargement of adipocyte resulting from increased lipid accumulation). Disruption of the mechanisms that control adipose tissue homeostasis can result in massive expansion of the tissue as exemplified during development of obesity (Henry et al., 2012). One of the most exciting challenges in the field of adipose tissue homeostasis is to identify the key regulators of adipose tissue expansion, which would allow a better understanding of the mechanisms involved in the pathogenesis of adipose tissue disorders. Recent reports based on functional studies and identification of novel enzymatic properties suggest that some AKR1B enzymes can regulate adipose tissue homeostasis.

Akr1b7: a negative regulator of adipose expansion

Until 2003, there was no mention of AR expression in white adipose tissue. In a transcriptomic study, Moraes et al. reported for the first time the detection of *Akr1b7* mRNA in mouse white adipose tissue. In this study, the authors highlighted a decrease in *Akr1b7* expression in abdominal white fat tissue from diet-induced obese mice when compared with mice fed a standard diet (Moraes et al., 2003). This decrease reflected either the down-regulation of *Akr1b7* gene expression level per cell or the lower proportion of cells expressing the gene. Indeed, *Akr1b7* expression appeared to vary depending on the location of white adipose depots and was found enriched in the stromal vascular fraction (containing adipocyte progenitors) whereas it was virtually absent from mature adipocytes (Tirard et al., 2007). Accordingly, *Akr1b7* expression decreased during adipogenic differentiation of primary preadipocytes from the stromal vascular fraction and was detectable in the 3T3-L1 preadipocyte cell line.

The transcription factor SF-1, an essential regulator of *Akr1b7* expression in the adrenal cortex is absent from adipose tissue. Instead, adipocyte precursors express liver receptor homolog-1 (LRH-1; Clyne et al., 2002), a nuclear receptor that also binds to SF-1 regulatory elements and can substitute for SF-1 in tissues where it is not expressed (Sirianni et al., 2002). Consistent with decreased expression of Akr1b7 during adipogenesis, LRH-1 expression is rapidly down-regulated during adipocyte differentiation (Clyne et al., 2002). We thus evaluated the possibility that LRH-1 stimulated *Akr1b7* promoter activity in preadipocytes. Indeed, in co-transfection experiments in 3T3-L1 preadipocytes, LRH-1 induced *Akr1b7* promoter through the SF-1 binding site at −458 (unpublished observations). Therefore, the dynamic expression profile of LRH-1 may account for expression of *Akr1b7* in the preadipocytes-enriched stromal fraction (stromal vascular fraction) of various fat depots and its transitory expression in the early differentiation steps of 3T3-L1 cells.

Overexpression of Akr1b7 in 3T3-L1 preadipocytes prevented lipid droplets accumulation during adipogenic differentiation. In contrast, knock-down of Akr1b7 accelerated differentiation and lipid accumulation in 3T3-L1 cells. These results allowed us to demonstrate that Akr1b7 is a negative regulator of adipogenesis *in vitro*, which prevents differentiation by reducing lipid storage (Tirard et al., 2007). The mechanisms through which Akr1b7 inhibits adipocyte differentiation *in vivo* were recently unraveled by our group using *Akr1b7*$^{-/-}$ mice. These will be discussed below.

Akr1b7 has two different enzymatic activities that could potentially be involved in the regulation of white adipose tissue homeostasis. First, just like Akr1b3, Akr1b7 is endowed with PGFS

activity allowing synthesis of $PGF_{2\alpha}$, a potent inhibitor of adipogenesis (Serrero et al., 1992; Kabututu et al., 2009). Indeed, $PGF_{2\alpha}$ is able to inhibit differentiation of 3T3-L1 into adipocytes, through binding to the FP receptor. Activation of FP receptor in turn blocks PPARγ and C/EBPα expression through a $G\alpha_q$-calcium-calcineurin-dependent signaling pathway (Liu and Clipstone, 2007). $PGF_{2\alpha}$ was also shown to block adipogenesis through activation of mitogen-activated protein kinase, resulting in inhibitory phosphorylation of PPARγ (Reginato et al., 1998). Second, Akr1b7 reduces 4-HNE, the accumulation of which is known to induce an excessive development of adipose tissue through adipocytes hypertrophy. Detailed analysis of *Akr1b7*$^{-/-}$ mice allowed us to show that Akr1b7 behaved as an anti-adipogenic factor limiting white adipose tissue expansion, essentially through the regulation of $PGF_{2\alpha}$ levels *in vivo* (Volat et al., 2012; **Figure 7**).

Akr1b7$^{-/-}$ mice displayed excessive basal adiposity resulting from both adipocyte hyperplasia and hypertrophy. They further exhibited increased sensitivity to diet-induced obesity. Following adipose enlargement and irrespective of the diet, they developed liver steatosis and progressive insulin-resistance. Akr1b7 loss was associated with decreased $PGF_{2\alpha}$ white adipose tissue contents. Cloprostenol (a $PGF_{2\alpha}$ agonist) administration in *Akr1b7*$^{-/-}$ mice normalized white adipose tissue expansion by altering both *de novo* adipocyte differentiation and size. Treatment of 3T3-L1 adipocytes and *Akr1b7*$^{-/-}$ mice with cloprostenol suggested that decreased adipocyte size resulted from inhibition of lipogenic gene expression. Hence, Akr1b7 is a major regulator of white adipose tissue development through at least two $PGF_{2\alpha}$-dependent mechanisms: inhibition of adipogenesis and lipogenesis (Volat et al., 2012).

Akr1b8/AKR1B10

Although we showed that Akr1b8 is devoid of PGH_2 9-,11-endoperoxide reductase activity (Kabututu et al., 2009), QTL mapping allowed identification of Akr1b8 as a possible regulator of adiposity in mouse (Derry et al., 2010). *Akr1b8*$^{-/-}$ male mice had a tendency to be fatter than their wild-type littermates, under both standard and high fat diets. This was particularly obvious for the gonadal fat pad. Under high fat diet, *Akr1b8*$^{-/-}$ mice also displayed higher serum cholesterol than their wild-type littermates. The mechanisms involving Akr1b8 in the regulation of white adipose tissue homeostasis remain completely unknown and are not necessarily resulting from expression of the gene in the adipose tissue. Indeed, we were unable to detect significant Akr1b8 protein expression in various adipose depots in mouse (Volat et al., 2012). Although we cannot exclude that Akr1b8 could be expressed in variable amounts depending on the mouse genetic background and the location of fat pads, it should be considered that the effect of Akr1b8 ablation could be indirect. Thus the involvement of Akr1b8 in adipose tissue homeostasis should be carefully examined with respect to its expression sites and enzymatic activities.

AKR1B10 expression in the adipose tissue has not yet been determined. Because AKR1B10 and Akr1b8 are not only detoxifying enzymes but also affect *de novo* fatty acids synthesis in cancer cells, it would be interesting to determine if and which of the

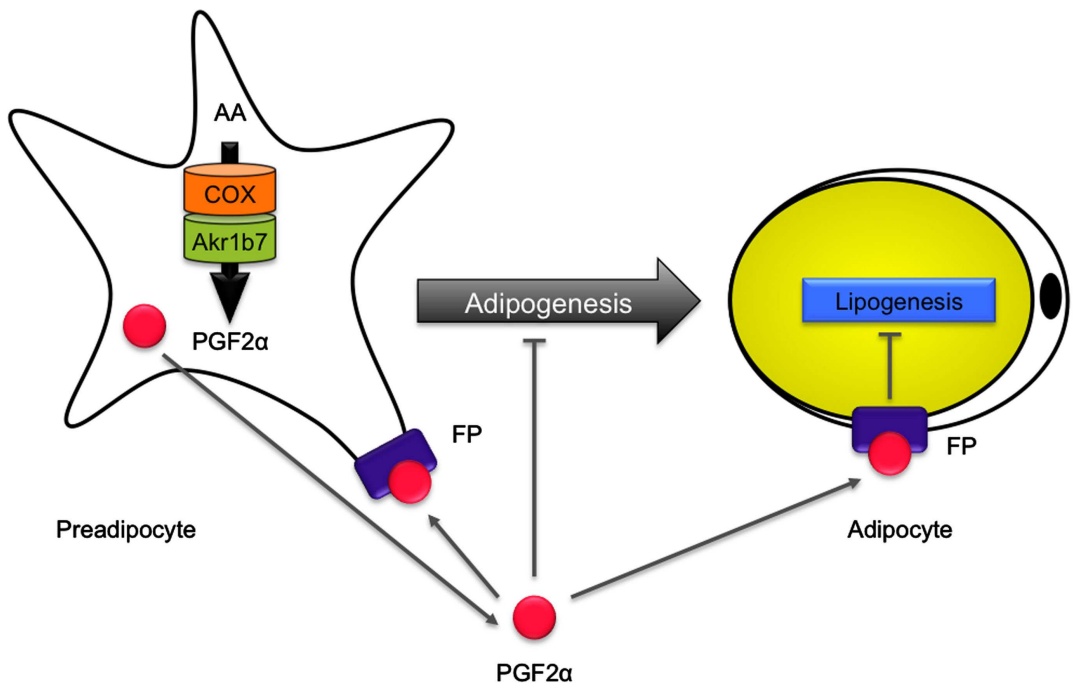

FIGURE 7 | Role of Akr1b7 in adipose tissue homeostasis. In preadipocytes, arachidonic acid (AA) is metabolized into PGH_2 by COX and is further converted into $PGF_{2\alpha}$ by the PGFS activity of Akr1b7. $PGF_{2\alpha}$ is supposed to mediate an autocrine activation of FP receptors on preadipocytes that inhibits their differentiation into adipocytes. In addition, $PGF_{2\alpha}$ inhibits lipogenesis in mature adipocytes. Accordingly, altered production of $PGF_{2\alpha}$ in *Akr1b7*$^{-/-}$ mice leads to an expansion of adipose tissue due to both hyperplasia and hypertrophy of adipocytes.

enzymatic activities of AKR1B10 could be involved in adipose tissue homeostasis (Ma et al., 2008; Wang et al., 2009; Joshi et al., 2010).

Akr1b3/AKR1B1

Akr1b3 is expressed in undifferentiated 3T3-L1 preadipocytes and during the early phase of their differentiation. Akr1b3 can synthesize $PGF_{2\alpha}$ in 3T3-L1 cells, which inhibits their differentiation through stimulation of the FP receptor (Fujimori et al., 2010).

In addition to their $PGF_{2\alpha}$ synthase activity, Akr1b3 and AKR1B1 also catalyze the isomerization of PGH_2 to PGD_2 (Nagata et al., 2011). The impact of PGD_2 on white adipose tissue homeostasis is still very disputed. Indeed, lipocalin-type prostaglandin D synthase (*L-PGDS*$^{-/-}$) mice show hypertrophy of adipocytes (Ragolia et al., 2005). In contrast, the knock-down of L-PGDS decreases lipid accumulation in 3T3-L1 cells (Fujimori et al., 2007). In agreement with these observations, transgenic mice overexpressing (human hematopoietic-type prostaglandin D synthase (H-PGDS) overproduce PGD_2 and show signs of obesity and pronounced adipogenesis on a high fat diet (Fujitani et al., 2010). These investigations suggest that PGD_2 promotes adipogenesis *in vivo*.

Because of its involvement in the synthesis of $PGF_{2\alpha}$ and PGD_2, the involvement of Akr1b3 in white adipose tissue physiology may result from a balance between both enzymatic activities. However the relevance of these data has not yet been studied *in vivo*. Indeed, no defect in adipose tissue homeostasis have been reported in *Akr1b3*$^{-/-}$ mice and the prostaglandin contents of their fat depots have not been analyzed (Aida et al., 2000; Ho et al., 2000).

Although the murine AR Akr1b3 could potentially participate in the homeostatic maintenance of adipose tissue, there is no available information about its human ortholog AKR1B1. Previous studies using transgenic mice with constitutive overexpression of AKR1B1 did not report any effect on adipose tissue (Yamaoka et al., 1995). On the basis of its enzymatic properties ($PGF_{2\alpha}$ and PGD_2 synthesis and reduction of 4-HNE), AKR1B1 could be considered as a potential actor of adipose tissue physiology (Kabututu et al., 2009; Nagata et al., 2011; Shen et al., 2011). However its expression pattern in human fat depots remains to be determined.

CONCLUSIVE REMARKS

Understanding physiological functions of AKR1B/Akr1b enzymes is a really challenging task. In that regard, review of the literature highlights the marked contrast between the abundance of enzymatic data and the small number of reports dedicated to functional studies in a physiological context. The main reason for this discrepancy is likely to be rooted in the functional redundancy resulting from the obvious structure conservation of the members of this enzyme family. Moreover, *Akr1b* genes being tandemly arranged on a same chromosome, classical genetic approaches to study multigene families that consist in combining KO models for each isoforms to obtain double or triple mutant mice are almost impossible to setup. Thus, alternative approaches to genetically disrupt *Akr1b* in various combinations and locations are mandatory to advance our understanding of their functions. Nuclease targeted invalidation or RNA interference approaches combined to existing KO models could present as valuable options.

Gain/loss-of-function models available for the Akr1b7 isoform revealed its implication in metabolic function and adipose tissue homeostasis. Beside the widely accepted idea that Akr1b are detoxification enzymes, several teams have now provided functional demonstration that some isoforms are endowed with the capacity to produce directly (prostaglandins) or indirectly (fatty acids) signal molecules. We think that further exploration of the functions of AKR1B/Akr1b enzymes must now take this dual potentiality into account.

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Aldose reductase, oxidative stress, and diabetic mellitus

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Diabetes mellitus (DM) is a complex metabolic disorder arising from lack of insulin production or insulin resistance (Diagnosis and classification of diabetes mellitus, 2007). DM is a leading cause of morbidity and mortality in the developed world, particularly from vascular complications such as atherothrombosis in the coronary vessels. Aldose reductase (AR; ALR2; EC 1.1.1.21), a key enzyme in the polyol pathway, catalyzes nicotinamide adenosine dinucleotide phosphate-dependent reduction of glucose to sorbitol, leading to excessive accumulation of intracellular reactive oxygen species (ROS) in various tissues of DM including the heart, vasculature, neurons, eyes, and kidneys. As an example, hyperglycemia through such polyol pathway induced oxidative stress, may have dual heart actions, on coronary blood vessel (atherothrombosis) and myocardium (heart failure) leading to severe morbidity and mortality (reviewed in Heather and Clarke, 2011). In cells cultured under high glucose conditions, many studies have demonstrated similar AR-dependent increases in ROS production, confirming AR as an important factor for the pathogenesis of many diabetic complications. Moreover, recent studies have shown that AR inhibitors may be able to prevent or delay the onset of cardiovascular complications such as ischemia/reperfusion injury, atherosclerosis, and atherothrombosis. In this review, we will focus on describing pivotal roles of AR in the pathogenesis of cardiovascular diseases as well as other diabetic complications, and the potential use of AR inhibitors as an emerging therapeutic strategy in preventing DM complications.

Keywords: aldose reductase, oxidative stress, diabetes mellitus, atherosclerosis, thrombosis

INTRODUCTION

In mammalian cells, under normoglycemia (3.8–6.1 mmol/L), cellular glucose is predominantly phosphorylated into glucose 6-phosphate by hexokinase, and enters the glycolytic pathway. Only trace amounts of non-phosphorylated glucose (about 3%) enter the polyol pathway (Morrison et al., 1970). However, under hyperglycemic condition (>7 mmol/L), there is increased flux through the polyol pathway, accounting for greater than 30% of glucose metabolism (Gonzalez et al., 1984; Yabe-Nishimura, 1998). The rate limiting step of the polyol pathway is the reduction of glucose to sorbitol catalyzed by aldose reductase (AR), at the expense of reduced nicotinamide adenosine dinucleotide phosphate (NADPH). Sorbitol is, in turn, converted to fructose by sorbitol dehydrogenase (SDH) with the oxidized form of nicotinamide adenine dinucleotide (NAD^+) as a co-factor (Yabe-Nishimura, 1998; El-Kabbani et al., 2004; **Figure 1**). The polyol pathway was first identified in the seminal vesicle by Hers (1956) who demonstrated the conversion of blood glucose into fructose, an energy source for sperm cells. AR has since been isolated and purified from a number of human and animal tissues including various regions of the eyes (Srivastava et al., 1984), testis (Kawasaki et al., 1989), liver (Petrash and Srivastava, 1982), placenta (Das and Srivastava, 1985a; Vander Jagt et al., 1990a), ovary (Iwata et al., 1990), kidney (Ansari et al., 1991; Ohta et al., 1991), erythrocyte (Das and Srivastava, 1985b), cardiac (Vander Jagt et al., 1990b) and skeletal muscle (Cromlish and Flynn, 1983; Morjana and Flynn, 1989; Vander Jagt et al., 1990b), and the brain (Wermuth et al., 1982; Cromlish et al., 1985). AR is located in the cytoplasm of most cells (Flynn, 1982) but is not uniformly distributed in all cell types of an organ. For example, in the kidney the enzyme is present in the Henle's loop, collecting tubules, outer and inner medulla, but not in the cortex (Terubayashi et al., 1989; Ohta et al., 1991).

CONTRIBUTION OF ALDOSE REDUCTASE TO DIABETES-INDUCED OXIDATIVE STRESS

Diabetes mellitus (DM) is characterized by chronic hyperglycemia and disturbances of carbohydrate, fat, and protein metabolism resulting from an absolute or relative deficiency of insulin (Diagnosis and classification of diabetes mellitus, 2007). Increased oxidative stress is thought to play an important role in the pathogenesis of diabetic complications, as supported by increased levels of oxidized DNA, proteins, and lipids (Wiernsperger, 2003). The induction of oxidative stress in DM can result from multiple mechanisms. Excessive levels of glucose can disrupt the electron transport chain in the mitochondria, leading to overproduction of superoxide anions (Nishikawa et al., 2000). High glucose can also stimulate oxidative stress via the auto-oxidation of glucose (Wolff and Dean, 1987) and through non-enzymatic glycation (Mullarkey et al., 1990). Reactive oxygen species (ROS) is generated in the process of advanced glycation endproducts (AGEs) formation (Kennedy and Lyons, 1997; Yim et al., 2001) and interaction between AGEs and their receptors RAGE can also lead to ROS production (Schmidt et al., 1994). Moreover, glycation can inactivate antioxidant enzymes, impairing antioxidant defense, as observed with glycation of superoxide dismutase (Kawamura et al., 1992; Morgan et al., 2002).

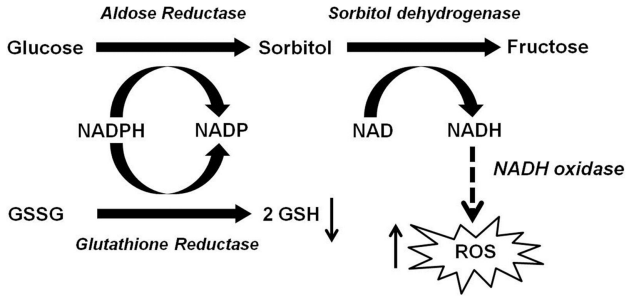

FIGURE 1 | Role of aldose reductase (AR) in hyperglycemia-induced oxidative stress. Excessive amount of glucose is shunted to the polyol pathway, where AR reduces glucose into sorbitol at the expense of NADPH. Since NADPH is essential for generation of GSH (intracellular antioxidant) from GSSG, the depletion of NADPH by the AR pathway may impair intracellular antioxidant defense. Sorbitol is then converted to fructose by SDH with the production of NADH, potentially leading to increased ROS via NADH oxidase.

Another important mechanism whereby high glucose can induce oxidative stress is the polyol pathway. Previous studies using AR deficient mice have shown that polyol pathway is an important source of diabetes-induced oxidative stress (Lee and Chung, 1999; Obrosova et al., 2003, 2005; Drel et al., 2006, 2008; Ho et al., 2006). There are three potential mechanisms by which the polyol pathway contributes to oxidative stress. First, under hyperglycemic condition, 30% of the glucose is channeled into AR-dependent polyol pathway, which depetes NADPH and consequently reduces GSH level (Cheng and Gonzalez, 1986). Second, oxidative stress is generated during the conversion of sorbitol into fructose by SDH (i.e., the second step of polyol pathway). In this step, the co-factor NAD^+ is converted to NADH by SDH. NADH is a substrate for NADH oxidase leading to production of superoxide anions (Morre et al., 2000). Third, the polyol pathway converts glucose to fructose, and fructose can be further metabolized into fructose-3-phosphate and 3-deoxyglucosone, which are more potent non-enzymatic glycation agent than glucose (Hamada et al., 1996a,b). Thus, the flux of glucose through the polyol pathway would increase AGEs formation, ultimately leading to ROS generation. Thus there is crosstalk between AR-dependent and AR independent sources of oxidative stress making it difficult to establish the relative contributions of each. Additionally, the pathways leading to production of oxidative stress is both tissue and cell dependent. Relative contributions of oxidative stress remains an outstanding question.

ALDOSE REDUCTASE AND ATHEROTHROMBOTIC CARDIOVASCULAR DISEASE IN DIABETES

Latest estimates predict that the global prevalence of DM will increase by 165% from 11 million in 2000 (prevalence of 4.0%) to 29 million in 2050 (prevalence of 7.2%; Boyle et al., 2001). Atherothrombotic cardiovascular events account for up to 80% of all deaths among DM patients (Haffner et al., 1998). While standard preventative treatments to combat atherothrombosis include glycemic control and low dose aspirin, challenges remain. The effectiveness of tight glycemic control has recently been the subject of considerable debate, with some studies suggesting that it is of marginal benefit in preventing cardiovascular events relative to less stringent glycemic control (American Diabetes Association, 2000, 2003; Wilson and Perry, 2009). While low dose aspirin is protective in many patients, some individuals exhibit aspirin-resistance (Grotemeyer, 1991; Grotemeyer et al., 1993; Helgason et al., 1994; Pappas et al., 1994; Buchanan and Brister, 1995; Marshall et al., 1997; Andersen et al., 2002; Macchi et al., 2002; Grundmann et al., 2003; Zimmermann et al., 2003). Therefore there is an urgent need for novel pharmacological agents to reduce the increasing burden of atherothrombotic cardiovascular disease.

The pathogenesis of the diabetic complications is complex with multiple mechanisms proposed, including (1) non-enzymatic glycation, (2) protein kinase C (PKC) activation, (3) hexosamine pathway activation, and (4) mitochondrial respiratory chain disruption. However, one of the major proposed mechanisms for the development of the diabetic complications involves the polyol pathway, which mediates the metabolic and osmotic alterations in many tissues (e.g., neurons, platelets). Increased glucose flux through the polyol pathway has been associated with the pathogenesis of diabetic complications via several potential mechanisms, including sorbitol-osmotic effects, depletion of myoinositol (Kinoshita et al., 1962) and subsequent perturbations in Na^+/K^+ ATPase activity (Greene et al., 1987; Steele et al., 1993), disturbances in cellular redox and free radical defense, increased oxidative, and glycation stress, activation of PKC (Steele et al., 1993; Hamada et al., 2000; Hamada and Nakamura, 2004), nitric oxide (NO)-mediated vascular tone (Tesfamariam et al., 1993), and induction of hyperglycemic pseudohypoxia (Van den Enden et al., 1995; **Figure 2**). Moreover, polymorphic markers of the human AR gene demonstrate a strong association with a susceptibility to develop diabetic complications. This suggests that the polyol pathway plays an important role in the pathogenesis of DM in human patients. Indeed a number of AR inhibitors are currently being investigated to prevent diabetic complications such as cardiomyopathy, neuropathy, nephropathy, and retinopathy (Johnson et al., 2004; Giannoukakis, 2006; Ramirez and Borja, 2008).

Diabetes has been viewed as a coronary heart disease and myocardial infarction risk equivalent, in part due to its association with a hypercoagulable state and elevated concentration of procoagulant factors, including fibrinogen and von Willebrand factor (Kessler et al., 1998; Boden and Rao, 2007). Even acute increases in blood glucose concentration cause spontaneous platelet aggregation, while AR inhibition significantly inhibits platelet aggregation (May et al., 1990), and has anti-platelet activity both *in vitro* and *in vivo* (Tawata et al., 1992), indicating a direct contribution to platelet aggregation. During chronic hyperglycemia, platelets from diabetic patients have increased responsiveness to collagen and adenosine diphosphate (ADP), which can be normalized by treatment with the AR inhibitor, sorbinil (Jennings et al., 1990). Previous animal studies also demonstrated that AR inhibition improved platelet hyperaggregation in streptozotocin-induced diabetic rats (Hara et al., 1995; Hotta et al., 1995). A recent proteomic study has shown that AR is abundantly expressed in human platelets, and its inhibitor, epalrestat, reduces platelet aggregation (Schulz et al., 2010), supporting a crucial role of AR in platelet aggregation. Consistent with these findings, inhibition of AR has also been demonstrated to attenuate the hyperglycemia-induced

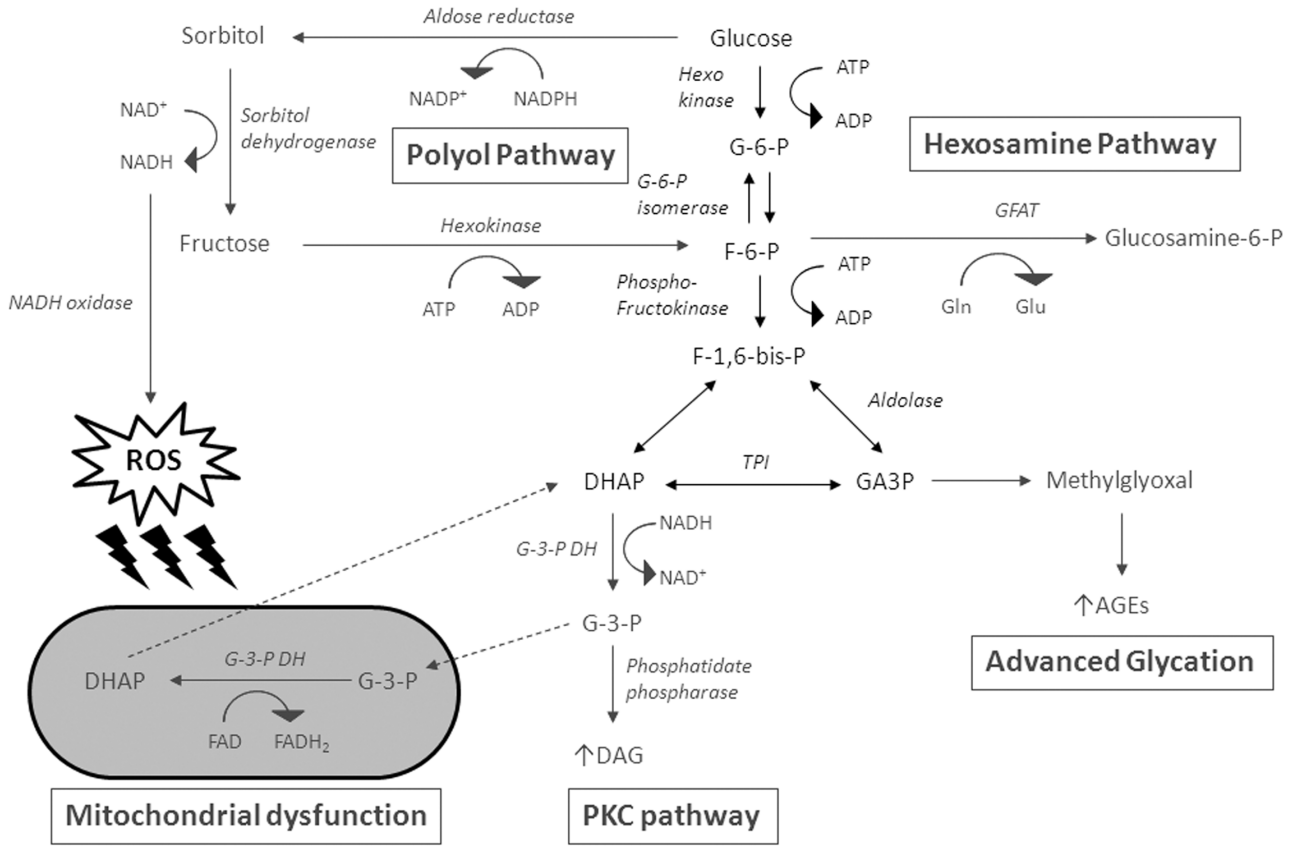

FIGURE 2 | Glucose flux through the polyol pathway has been associated with the pathogenesis of diabetic complications via several potential mechanisms. Intracellular accumulation of sorbitol causes osmotic stress. The end production of the polyol pathway, fructose, is converted to fructose-6-phosphate (F-6-P) by hexokinase, and is further converted to glucosamine-6-phosphate by glutamine: fructose-6-phosphate amidotransferase (GFAT). Fructose-6-phosphate may also form fructose-1,6-bisphosphate (F-1,6-P), which is converted to dihydroxyacetone phosphate (DHAP). DHAP and glyceraldehdye-3-phosphate (GA3P) are interconvertible by triosephosphate isomerase. They can lead to the formation of methylglyoxal, resulting in advanced glycation end-product. DHAP can further be converted to diacylglycerol (DAG), leading to PKC activation. The continuous conversion of glycerol-3-phosphate (G-3-P) to DHAP results in concomitant transfer of electrons from reduced cytosolic NADH to mitochondrial oxidized FAD, which can generate high mitochondrial membrane potentials and inhibition of the electron transport chain at complex III. The oxidation of NADH by NADH oxidase produces reactive oxygen species (ROS), which can attack the mitochondrial membrane.

platelet hyperaggregation in human platelet by reducing oxidative stress (Tang et al., 2011). All these findings suggest that AR plays a central role in platelet aggregation, particularly during hyperglycemic conditions. Oxidative stress generated by the AR-dependent polyol pathway likely plays a major role in diabetic platelet hyperaggregation.

Interestingly, generalized overexpression of human AR in diabetic mice demonstrated increased expression of inflammatory markers and uptake of modified lipoprotein in macrophages. This AR overexpression increases atherosclerosis on a low-density lipoprotein receptor knockout background; a relatively low endogenous AR expression is found in wild-type mice (Vikramadithyan et al., 2005). Another study in ApoE$^{-/-}$ mice also demonstrated that human AR expression is proatherogenic and that expression, specifically in endothelial cells, leads to more severe disease (Vedantham et al., 2011). AR also contributes to diabetes abnormalities in vascular smooth muscle cell growth by increasing the intracellular oxidative stress, translocation, and phosphorylation of signaling targets (e.g., PKC) as well as release of TNF-α and related cytokines (Ramana et al., 2005; Srivastava et al., 2006; Reddy et al., 2009). Hyperglycemia-stimulated release of TNF-α and related cytokines from VSMCs might potentially mediate diabetes-induced acceleration of atherogenesis and endothelial dysfunction in humans. These data suggest that AR plays a critical role in atherothrombotic cardiovascular disease, and hyperglycemia in diabetic patients provides sufficient substrate for the vasculotoxic effects of this enzyme.

Besides diabetic vasculopathy, AR has also been found to play an important role in diabetic cardiomyopathy, characterized by myocardial contractile dysfunction independent of coronary artery disease (Rubler et al., 1972). A study using mouse hearts demonstrated that the activity of AR was increased (but its gene expression was suppressed) during the early stage of diabetes (Iwata et al., 2007). Despite low abundance of AR in mouse hearts,

it is believed that the increased AR activity (as with hyperglycemia) may exacerbate myocardial dysfunction, leading to diabetic cardiomyopathy. AR may lead to hyperosmotic stress and may induce cardiac myocyte apoptosis (Galvez et al., 2003). Recently, the activity of AR was found to increase NADH/NAD^+ ratio in diabetic rat heart, and inhibition of AR in diabetic hearts lowered the NADH/NAD^+ ratio, normalizing the response to glucose metabolism and improving cardiac function (Ramasamy et al., 1997). Furthermore, the AR inhibitor, fidarestat, has been shown to improve contractile dysfunction and normalize Ca^{2+} signaling in the hearts of diabetic *db/db* obese mice. The intracellular superoxide induced by diabetes was also attenuated by treatment with fidarestat, suggesting that the polyol pathway activity contributes to contractile dysfunction by increasing superoxide formation in cardiac myocytes under hyperglycemic condition (Dong and Ren, 2007).

ALDOSE REDUCTASE AND MYOCARDIAL ISCHEMIA/REPERFUSION INJURY

Myocardial ischemia/reperfusion (I/R) injury is one of the major causes of morbidity and mortality in patients with DM. Previous studies have indicated that ROS formed in the ischemic heart activate AR by modifying its cysteine residues to sulfenic acids (Kaiserova et al., 2008). Increased activity of AR in I/R rat hearts depletes intracellular NADPH, thereby reducing cellular GSH levels, increasing oxidative stress, as NADPH is also needed for the activity of glutathione reductase. AR was also reported to act as a mediator of late phase ischemic preconditioning. The increased AR activity at 24 h after ischemic preconditioning reduced the formation of HNE and the accumulation of HNE-modified proteins during myocardial I/R (Shinmura et al., 2002). Thus, a complete picture concerning the role of AR during myocardial ischemia remains elusive.

In recent years, it has been shown that AR is a key component of I/R injury in diabetic as well as non-diabetic heart (Ramasamy et al., 1997; Hwang et al., 2004). The protective mechanism contributed by AR inhibition is thought to be due to the preservation of high-energy phosphates and maintenance for a lower cytosolic NADH/NAD^+ ratio, which can prevent the depletion of ATP and redox imbalance during myocardial I/R. Further studies showed that AR mediated the myocardial I/R injury in mice by depleting the ATP level thus increasing ROS generation (Iwata et al., 2006). Oxidative stress generated by AR is believed to be in part contributed to by enhanced mitochondrial permeability transition pore openings (Ananthakrishnan et al., 2009). Moreover, the AR-dependent polyol pathway was also found to contribute to myocardial contractile dysfunction and tissue damage by increasing oxidative stress in I/R rat hearts (Tang et al., 2008, 2010). Therefore, it is believed that the pharmacological inhibition of AR presents a novel adjunctive approach for protecting ischemic hearts in both diabetic and non-diabetic patients.

Apart from AR, SDH (converting sorbitol to fructose) has also been found to be another novel target for adjunctive protection of the ischemic myocardium. Studies indicate that inhibition of SDH attenuated the increased cytosolic NADH/NAD^+ ratio and increased glycolysis as well as glucose oxidation (Hwang et al., 2003). This further supported the role of the polyol pathway in myocardial I/R injury, and suggests a mechanism for SDH competing with glyceraldehyde 3-phosphate dehydrogenase (GAPDH) for NAD^+. Thus, AR and SDH are both potential targets for pharmacological intervention for myocardial I/R injury.

ALDOSE REDUCTASE AND OTHER COMPLICATIONS IN DIABETES

The pathogenic role of AR in diabetes is not limited to cardiovascular complications, and similar mechanisms are also involved in other complications, such as retinopathy, nephropathy, and neuropathy. The AR-dependent polyol pathway plays a major role in diabetic cataractogenesis. Previous studies showed that structurally diverse AR inhibitors prevented cataract formation in streptozotocin-induced diabetic rats (Sun et al., 2006; Drel et al., 2008). The key role for AR in diabetic cataractogenesis is further supported by studies in AR-overexpressing mice. Sugar cataracts form in transgenic diabetic mice expressing human AR in the lens, but not in wild-type streptozotocin-induced diabetic mice which have very low expression of AR (Varma and Kinoshita, 1974; Lee et al., 1995). AR siRNA transfection and inhibition suppressed high glucose-induced ROS formation, NF-kappaB activation, and apoptosis in rat lens epithelial cells (Nambu et al., 2008). Studies on slow cataract formation showed that metabolic imbalance caused by increased AR activity plays a major role in slow cataract development in mature diabetic animals (Sun et al., 2006; Drel et al., 2008), which is more relevant to diabetic patients. Two further studies suggested an important role for AR in high glucose- and diabetes-induced impairment of lenticular signaling (Ramana et al., 2003; Zatechka et al., 2003). Therefore, increased AR activity is likely to contribute to diabetic cataract formation through oxidative signaling mechanisms.

The AR-dependent polyol pathway is one of the more promising targets for diabetic neuropathy. Increased AR activity leads to more severe diabetic neuropathy (Yagihashi et al., 2001; Song et al., 2003) and decreased levels of GSH (Song et al., 2003). Previous studies demonstrated that hyperglycemia-induced oxidative stress led to the activation of mitogen-activated protein kinase (MAPK), which may have contributed to neuronal pathogenesis (Wang et al., 1998; Purves et al., 2001). Fidarestat, an AR inhibitor, was shown to prevent activation of MAPK and nerve conduction velocity deficits in diabetes (Price et al., 2004), indicating that AR inhibitors could reduce the diabetes-induced oxidative stress. Other studies using AR knockout mice (Ho et al., 2000) also demonstrated that AR deficiency could prevent diabetes-induced oxidative stress in nerve cells in the retina (Cheung et al., 2005). Moreover, both AR deficiency and AR inhibition reduced oxidative stress in the peripheral nerves and markedly protected mice from diabetes-induced functional deficits (Ho et al., 2006). All these findings suggest that AR contributes to the pathogenesis of diabetic neuropathy via oxidative stress.

AR is differentially expressed in mammalian kidney, where AR expression is low under physiological condition in the glomerulus but significantly increased in diabetic human patients (Corder et al., 1979; Kasajima et al., 2001). In diabetic rats, it was found that hyperglycemia-induced increase in glomerular sorbitol levels was attenuated by treatment with an AR inhibitor, sorbinil (Beyer-Mears et al., 1984). Hyperactivation of AR in renal cells have been

linked with aberrant activation of PKC (Ishii et al., 1998; Kapor-Drezgic et al., 1999; Noh and King, 2007), generation of advanced glycation products, increased expression of TGF-β and generation of ROS (Oates and Mylari, 1999). A recent study using mice with AR deficiency in all tissues except in the renal medulla, showed that genetic ablation of AR significantly ameliorates the development of diabetic nephropathy in streptozotocin-induced diabetic mice (Liu et al., 2011). Together these data suggest that activation of AR by hyperglycemia in the renal glomeruli contributes to the onset and progression of diabetic nephropathy via oxidative stress.

SUMMARY

Accumulating evidence in experimental studies has demonstrated the mechanistic role of AR in various metabolic diseases associated with diabetes and its complications. Although a number of AR inhibitors have been tested or are currently undergoing testing in clinical trials (reviewed in Giannoukakis, 2008), the clinical efficacy is uncertain and there are concerns with associated adverse effects such as hepatic damage. One of the possible reasons for the discrepancy between experimental animal studies and human clinical studies (besides species differences) is the length of time between the onset of diabetes and start of the AR inhibitor treatment. In many experimental studies, treatment with AR inhibitors are often commenced before the onset of diabetic complications and induction of AR. In contrast, treatment with AR inhibitors are usually administrated to patients with longstanding DM where the affected tissues (e.g., nerves and retina) have already undergone extensive damage. Thus it is not surprising that the clinical efficacy of AR inhibitors is relatively low. However, human platelets (a critical contributor to atherothrombosis in DM) has a short life span with a high turnover rate and thus may respond to AR inhibitor therapy in conjunction with low dose aspirin. As discussed in this review, under hyperglycemic conditions, activation of the AR pathway upregulates many other glucose toxicity pathways (e.g., non-enzymatic glycation, PKC pathway, hexosamine pathway, and disruption of mitochondrial respiratory chain), so treatment with AR inhibitors alone may not be as effective. AR inhibitors may serve as an effective adjunct therapy for prevention of diabetic complications.

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The prostaglandin F synthase activity of the human aldose reductase AKR1B1 brings new lenses to look at pathologic conditions

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Prostaglandins are important regulators of female reproductive functions to which aldose reductases exhibiting hydroxysteroid dehydrogenase activity also contribute. Our work on the regulation of reproductive function by prostaglandins (PGs), lead us to the discovery that AKR1B5 and later AKR1B1were highly efficient and physiologically relevant PGF synthases. PGE2 and PGF2α are the main prostanoids produced in the human endometrium and proper balance in their relative production is important for normal menstruation and optimal fertility. Recent evidence suggests that PGE2/EP2 and PGF2α/FP may constitute a functional dyad with physiological relevance comparable to the prostacyclin-thromboxane dyad in the vascular system. We have recently reported that AKR1B1 was expressed and modulated in association with PGF2α production in response to IL-1β in the human endometrium. In the present study, we show that the human AKR1B1 (gene ID: 231) also known as ALDR1 or ALR2 is a functional PGF2α synthase in different models of living cells and tissues. Using human endometrial cells, prostate, and vascular smooth muscle cells, cardiomyocytes and endothelial cells we demonstrate that IL-1β is able to up regulate COX-2 and AKR1B1 proteins as well as PGF2α production under normal glucose concentrations. We show that the promoter activity of AKR1B1 gene is increased by IL-1β particularly around the multiple stress response region containing two putative antioxidant response elements adjacent to TonE and AP1. We also show that AKR1B1 is able to regulate PGE2 production through PGF2α acting on its FP receptor and that aldose reductase inhibitors like alrestatin, Statil (ponalrestat), and EBPC exhibit distinct and characteristic inhibition of PGF2α production in different cell models. The PGF synthase activity of AKR1B1 represents a new and important target to regulate ischemic and inflammatory responses associated with several human pathologies.

Keywords: prostaglandins, biosynthesis, regulation, endometrium, menstrual cycle

INTRODUCTION

The human aldose reductase ALR2 gene AKR1B1 is a notorious enzyme which has been associated with complications of diabetes for more than four decades (Srivastava et al., 2005). AKR1B1 is considered as the rate limiting enzyme of the polyol pathway responsible for the conversion of glucose into sorbitol. The association of AKR1B1 with cardiovascular risks, neuropathies, and nephropathies characteristic of diabetes is receiving increased acceptance (Alexiou et al., 2009). However, the contribution of the polyol pathway to the same complications is less convincing (Del Corso et al., 2008). Indeed, while many factors including inflammation induce increased expression and activity of AKR1B1, this enzyme has very little affinity for glucose at physiological concentrations and a detoxification action involving reduction of lipid peroxides has been proposed (Srivastava et al., 2005). While searching for the enzyme responsible for the production of PGF2α in the endometrium, we made the serendipitous and unexpected discovery that AKR1B5 (Madore et al., 2003) and later the human aldose reductase AKR1B1 (Bresson et al., 2011) were highly functional PGFS. Prostaglandins (PGs) are notorious mediators of pain and inflammation also associated with proliferation of cancer cells. While the same mediators are recognized as primary regulators of female reproductive function (ovulation, uterine receptivity, implantation, and parturition) they also contribute to occurrence of endometrial carcinomas, menorrhagia, dysmenorrhea, endometriosis, and premature labor. Among the different PGs, PGE2 and PGF2α are the main prostanoids produced in the human endometrium (Smith and Kelly, 1988; Sales and Jabbour, 2003a). PGs are synthesized from arachidonic acid (AA) and converted to PGG_2 and PGH_2 by PGH synthases (PGHS), also known as cyclooxygenases (COX). There are two documented isoforms of PGHS in human, the constitutive COX-1 and the inducible COX-2 encoded by two distinct genes (Smith et al., 1996). PGH_2 produced by COXs is the common precursor of all PGs generated by specific terminal synthases such as PGF synthase for PGF2α and PGE synthase for PGE2. The human terminal synthases responsible

for the biosynthesis of PGE2 (Park et al., 2006), PGD2 (Zhou et al., 2010), and PGI2 (Wu and Liou, 2005) are well characterized, but only little is known for the enzyme responsible for PGF2α production. Before our identification of the PGF synthase activity of aldo-ketoreductases (AKR) of the 1B family (Madore et al., 2003; Bresson et al., 2011) only one AKR of the 1C family, AKR1C3 was recognized as a functional PGF synthase in the human (Suzuki-Yamamoto et al., 1999).

PGF2α is a biologically active prostanoid belonging to the eicosanoid family of bioactive lipids (Narumiya and FitzGerald, 2001). Its biosynthesis occurs via different pathways involving reduction of PGH2 by a 9,11-endoperoxyde reductase (Watanabe, 2002). Several PGFS have been identified in animals (Madore et al., 2003), but until recently, AKR1C3 was the only isoform currently identified in human (Komoto et al., 2006). In the bovine endometrium, using a cell-free system, we have demonstrated a strong PGFS activity of purified recombinant AKR1B5 recently renamed as *bos taurus* AKR1B1 (Gene ID: 317748) as well as circumstantial association between its pattern of expression and PGF2α production. This represents a new putative function for this enzyme previously known for its 20α-HSD and glucose metabolism activities (Madore et al., 2003). The human and bovine AKR1B1 (EC:1.1.1.21) both belong to the AKR1B family and share 86% identity or homology. The human AKR1B1 (Gene ID: 231) also known as the aldose reductase or ALDR1belongs to the AKR superfamily composed of 140 members divided into 15 families (Jin and Penning, 2007). AKR1B1 (EC:1.1.1.21) is primarily known as the rate limiting enzyme for conversion of glucose to sorbitol in the polyol pathway, but recent studies revealed its ability to convert a wide array of substrates including aldehydes generated during lipid peroxidation and their glutathione (GSH) conjugates, phospholipids, atherogenic lipids, and steroids (Srivastava et al., 2005).

After identifying the bovine AKR1B1 as a potential PGFS (Madore et al., 2003), we have shown circumstantial association with PGF2α production in human endometrial cell lines (Chapdelaine et al., 2006) and in decidualized stromal cells (Kang et al., 2006). Later, the PGFS activity of mouse AKR1B3 and AKR1B7 as well as the human AKR1B1 were tested and confirmed using purified recombinant proteins in a cell-free system *in vitro* (Kabututu et al., 2009). Using a similar approach complemented with gain and loss of function in endometrial cell lines, we have demonstrated that the AKR1B1 was a functional PGFs in the human endometrium (Bresson et al., 2011). In the present study, we have investigated further the regulation of PGF2α production by different stimulators and inhibitors as well as the promoter cis-elements responsible for IL-1β response in human endometrial cell lines. Finally, we have studied the association between AKR1B1 and PGF2α production in representative tissues where PGF2α was found to exert physiological action *in vivo*. We have evaluated the potential of different AKR1B1 inhibitors (ARI) to alter PGs production in living cells and in fresh endometrial tissues under normal and high glucose conditions.

MATERIALS AND METHODS

REAGENTS

RPMI 1640 culture medium without phenol, Superscript II reverse transcriptase, TRIzol, lipofectamine 2000, and pCR3.1 vectors were purchased from Invitrogen (Life technologies, ON, Canada). TAQ DNA polymerase and buffer used for polymerase chain reaction were from NEB (New England Biolabs, ON, Canada). RiboMax polymerase kit for siRNAs was purchased from Promega (Madison, WI, USA). Qiaquick gel extraction kit and TA cloning pDrive vector were from Qiagen (Mississauga, ON, Canada). All oligonucleotide primers were chemically synthesized by Integrated DNA Technologies (IDT) (Coralville, IA, USA). [α-^{32}P]dCTP radioactivity was bought from PerkinElmer Life Sciences (Markham, ON, Canada). Bright Star-Plus nylon membrane and UltraHyb solution were purchased from Ambion Inc. (Austin, TX, USA). The rabbit COXs antibodies were kindly provided by Dr S Kargman (Merck, QC, Canada), rabbit anti-Phospho-cPLA$_2$ (Ser505) was from Santa Cruz biotechnology (Santa Cruz, CA, USA) and AKR1C3 was from Abcam Inc. (Cambridge, MA, USA). Goat anti-rabbit horse-radish peroxidase-conjugated IgG was bought from Jackson Immunoresearch Laboratories (West Grove, PA, USA). The Western Lightning™Chemiluminescence Reagent ***Plus*** was purchased from PerkinElmer (Woodbridge, ON, Canada). Arachidonic acid (AA), and AL-8810 were from Cayman Chemicals (Ann Arbor, MI, USA) and recombinant human IL-1β was purchased from Research and Diagnostic Systems (Minneapolis, MN, USA). Aldose reductase inhibitors (ARIs), [ponalrestat (Statil), Alrestatin, and EBPC] were from Tocris Bioscience (Ellisville, MO, USA).

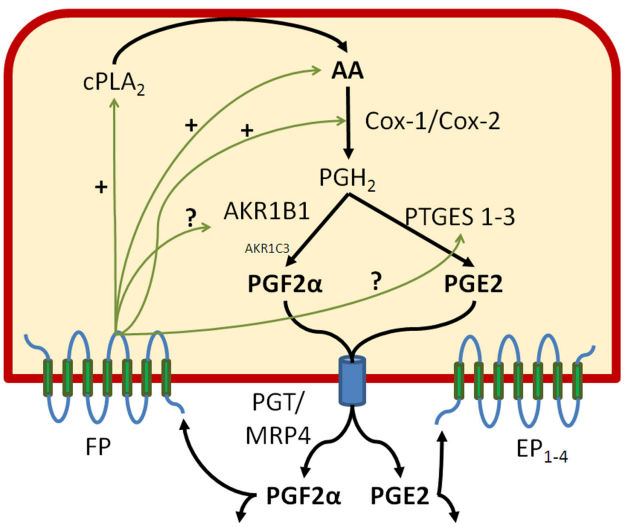

FIGURE 1 | Prostaglandin F2α and E2 biosynthetic pathways. In order to generate PGF2α, AKR1B1 must team up with other constitutive and inducible enzymes involved in the production of PGH2, the common precursor of all PGs. Among those, we have found that cPLA2 liberating AA from membrane phospholipids, COX-2, AKR1B1, and mPGES-1 are inducible by the cytokine IL-1β. AKR1B1 apparently works with both COX-1 and COX-2 producing PGH2 to generate PGF2α whereas mPGES-1 preferentially associates with COX-2 to generate PGE2. Both epithelial and stromal cells exhibit PGF2α responsiveness and stimulation or inhibition of the FP receptor alters the production of both PGE2 and PGF2α through action at several steps of the PG biosynthesis cascade. We also present in this figure the PG transporters PGT also known as solute carrier SLCOA1 and MRP4 the multidrug resistance protein 4 or ABCC4.

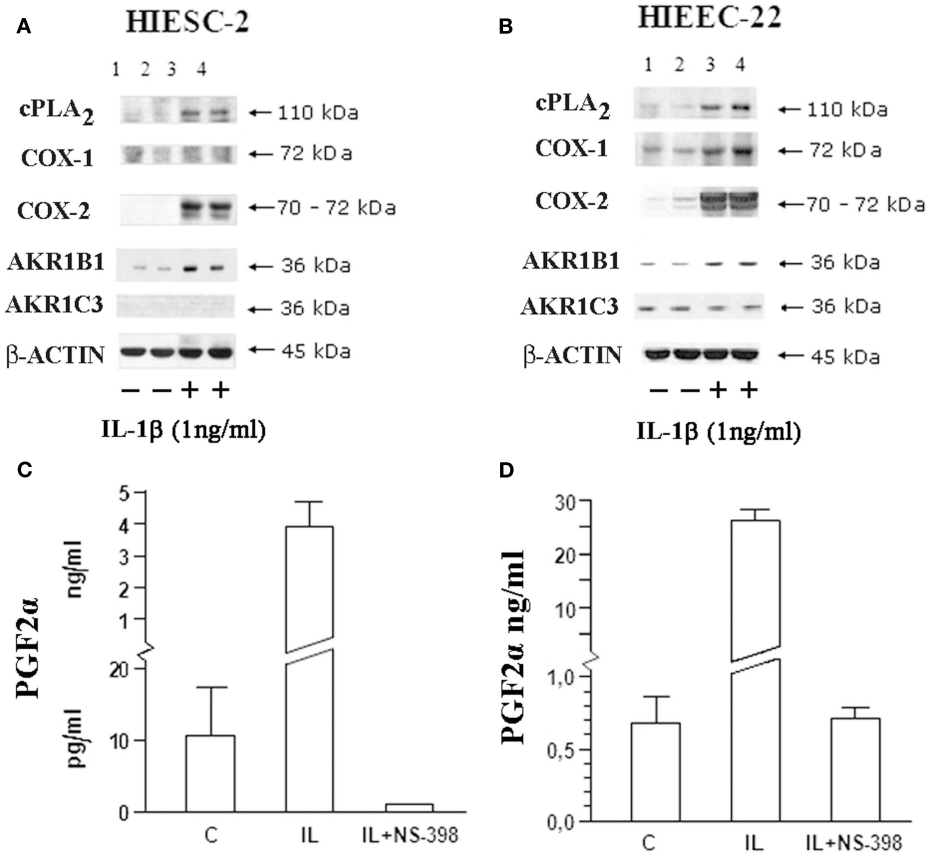

FIGURE 2 | Influence of IL-1β on the main biosynthetic enzymes associated with PGF2α production in human endometrial stromal and epithelial cells. Human endometrial stromal (HIESC-2) and epithelial (HIEEC-2) cells were grown to confluence and treated with IL-1β for 24 h in presence and absence of NS-398 to block COX activity. PGs biosynthetic enzymes were analyzed by Western blot [HIESC-2 **(A)**; HIEEC-22 **(B)**] and associated with PGF2α production HIESC-2 **(C)** and HIEEC-22 **(D)**.

CULTURED CELL MODELS

Human endometrial stromal (HIESC-2) and epithelial (HIEEC-22) cell lines were immortalized using SV40 large T antigen (Chapdelaine et al., 2006) and shown to exhibit most characteristics of freshly isolated endometrial cells (Kang et al., 2004, 2006). Bovine endometrial epithelial cells were immortalized using a similar protocol and also presented most characteristics of freshly isolated cells (Krishnaswamy et al., 2009, 2010; Lacroix-Pepin et al., 2011). Human prostate smooth muscle cells, human artery smooth muscle cells, human umbilical vein endothelial cells (Huvec), and human cardiomyocytes cells were from ScienCell Research Laboratories (Carlsbad, CA, USA).

NORTHERN BLOT

Northern blot analysis was performed as follows: total RNA (∼20 μg) was extracted with Trizol (Invitrogen, Life technologies, ON, Canada) from cultured endometrial cells, loaded on a 1.2% formaldehyde-agarose gel and electrophoresed at 100 V in 1× MOPS buffer. After electrophoresis, RNA was transferred overnight onto a nylon membrane in 10× saline-sodium citrate (SSC). The AKR1B1, mPGES-1, COX-1, and COX-2 cDNA probes were produced by digestion with restriction enzymes of different plasmids containing the corresponding cDNAs previously generated in our laboratory, each releasing a ∼500 bp fragment. The resulting probes were labeled with [α-^{32}P(dCTP (3000 Ci/mmol) using the Ready-To-Go DNA labeling Kit (GE Healthcare Life Science, QC, Canada). Prehybridization (2–4 h) and hybridization (overnight) were done at 45°C using Ultra-Hyb solution (Ambion Inc., Invitrogen, Life technologies, ON, Canada). The blots were then washed at 65°C twice for 15 min in 0.5 × SSC and exposed for 24–48 h at −80°C on BioMAx films PerkinElmer (Woodbridge, ON, Canada) to quantify the hybridization signal intensity. Finally, 18S ribosomal RNA stained with ethidium bromide was used to confirm uniform loading of RNA samples and a 32P-labeled oligonucleotide specific to 18S ribosomal RNA was used as an internal standard for Northern blot analysis.

WESTERN BLOT

Western blot analysis was performed as we described (Chapdelaine et al., 2006). Briefly, total proteins (∼20 μg) extracted from culture cells were loaded in each lane and electrophoresis done on 10% SDS-PAGE followed by electrotransfer onto nitrocellulose membrane (Bio-Rad laboratories, Mississauga, ON, Canada). The

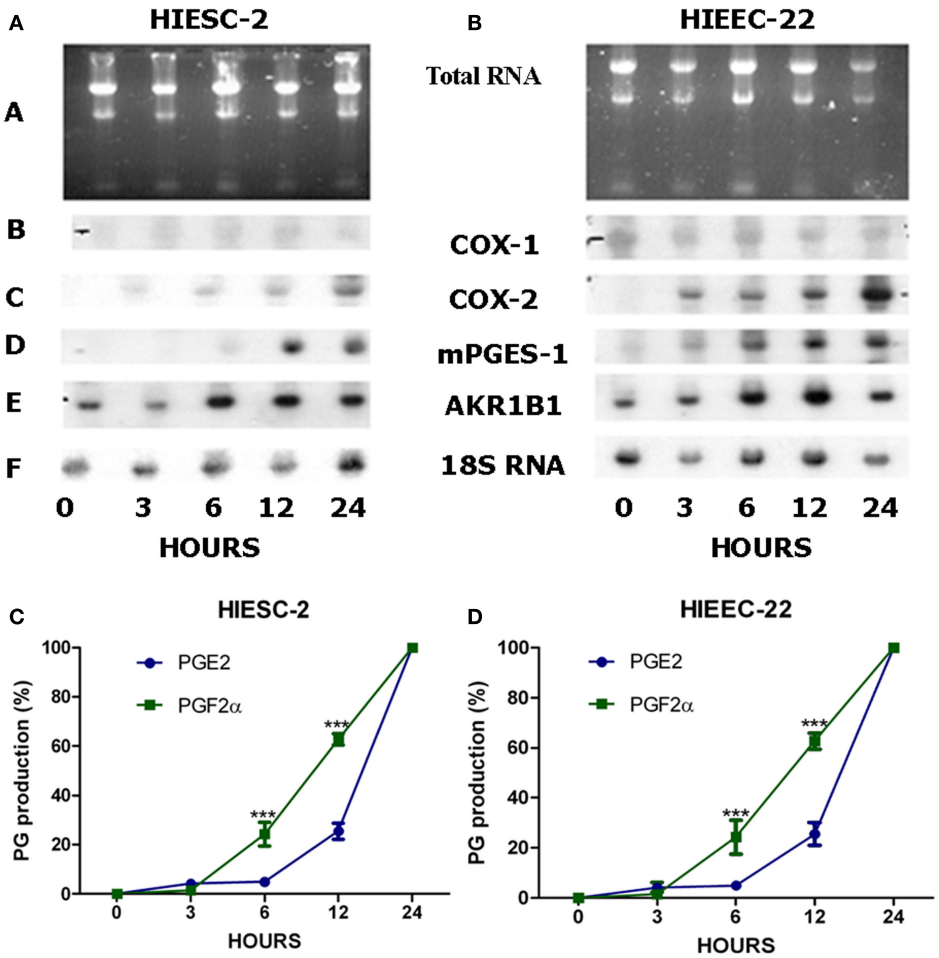

FIGURE 3 | Time course of gene expression and PGs production in human endometrial stromal and epithelial cells following stimulation with IL-1β. Human endometrial stromal (HIESC-2) and epithelial (HIEEC-2) cells were grown to confluence and treated with IL-1β for different periods up to 24 h. Northern Blots analysis **(A,B)** was used for detection of AKR1B1, COX-1/-2, mPGES-1 mRNAs expression. The production of PGs was evaluated as a function of time in stromal **(C)** and epithelial **(D)** endometrial cells where stimulation ofPGF2αprecedes that of PGE2 ($P < 0.05$).

primary antibodies used for the present study were the rabbit AKR1B1 (dilution 1/1000), COX-1 or COX-2 (dilution 1/3000), cPLA2 (1/1000) and goat AKR1C3 (1/500) anti-serums. As an internal control, the β-actin monoclonal antibody (1/5000, Sigma, Mississauga, ON, Canada) was used. The goat anti-rabbit IgG conjugated with horse radish peroxidase (HRP), rabbit anti-goat IgG HRP or goat anti-mouse IgG HRP were used as secondary antibodies. The chemiluminescence was analyzed with autoradiography films at optimal times of exposure following treatment of the membranes with the Western Lightning™Chemiluminescence Reagent *Plus (PerkinElmer Canada)*.

ANALYSIS OF AKR1B1 PROMOTER ACTIVITY

A nested PCR strategy was developed to generate a long ∼4.5 kb promoter to study the regulation of the AKR1B1 gene (AF032455). The promoter PCR fragments were amplified with PFU turbo DNA polymerase (Stratagene, Agilent technologies, USA) and cloned directly by fusion (Clontech, TAKARA Biocompany) in a pGL3 basic vector (Promega) containing the firefly luciferase gene. Constructs of AKR1B1 promoter of seven different lengths were generated using restriction enzymes targeting unique sequences in the long promoter. A short portion of the AKR1B1 promoter corresponding to the multiple stress response region (MSRR) −1710 to −1064 to the translation initiation ATG position was amplified by PCR, cloned in a TA cloning vector (pDrive, Qiagen) for sequencing and linked in a pGL3 vector (Promega) to the minimal SV40 promoter. From this, additional constructs were produced by deletion of the cis-elements corresponding to TONE and AP1 or ARE1 and 2. Stromal (HIESC-2) and epithelial (HIEEC-22) cells were co-transfected with the lipofectamine 2000 transfection agent (Invitrogen) and the different plasmid constructs possessing the firefly luciferase reporter and a plasmid containing the synthetic Renilla luciferase gene. After transfection, cells were treated for 24 h with IL-1β (1ng/ml), and the promoter activity was expressed as the ratio of firefly luciferase to the renilla luciferase using the Dual-Luciferase Reporter (DLR™) Assay System (Promega, Madison, WI, USA) using a Luminoskan Ascent luminometer (ThermoElectron Corporation, Milford, MA, USA).

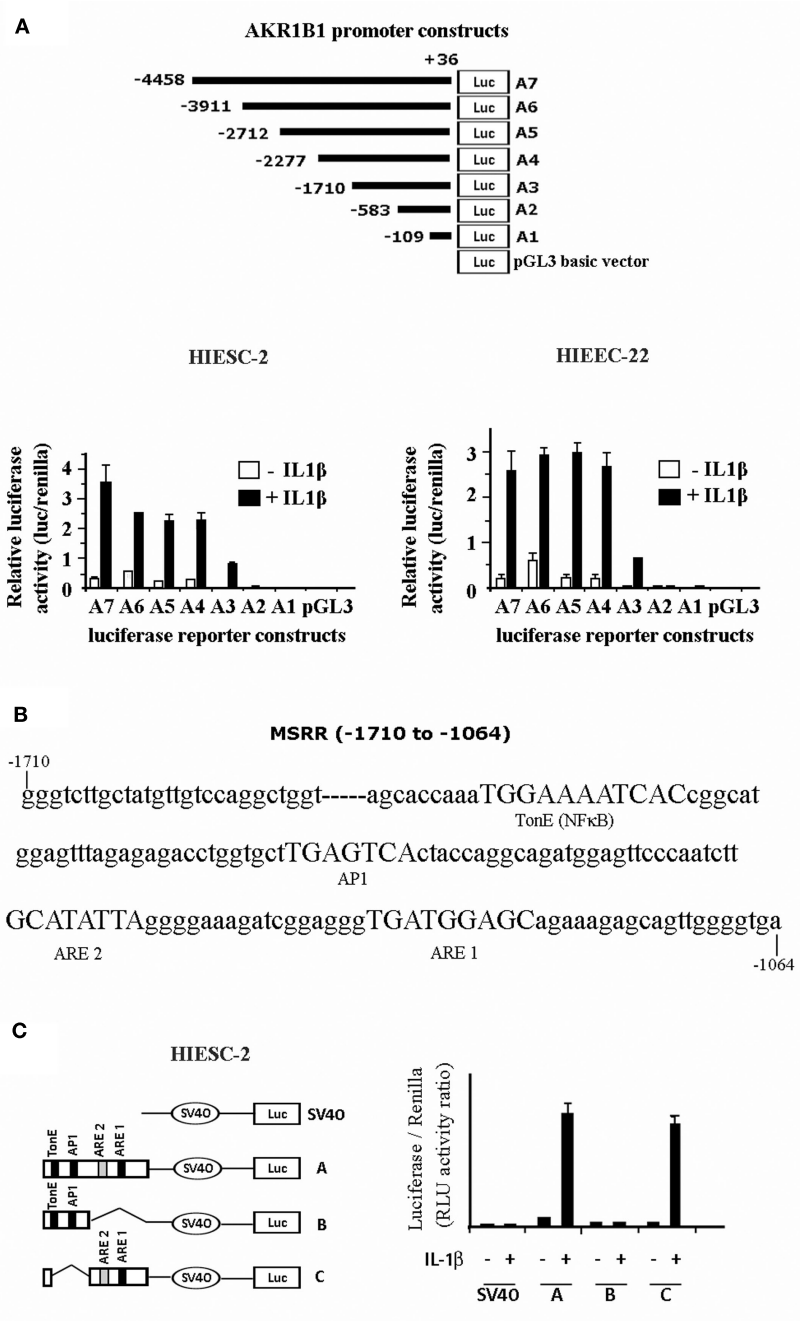

FIGURE 4 | Analysis of AKR1B1 promoter activity involved in IL-1β response in human endometrial cells. (A) Schematic representation of different AKR1B1 promoter constructs and corresponding activity following treatment of transfected stromal and epithelial cells treated with IL-1β. **(B)** Nucleotide sequence of the AKR1B1 promoter MSRR region (−1710 to −1064) showing the organization of the important cis-elements TonE (NFκB), AP1 and anti-oxidant response elements 1 and 2 (ARE1 and ARE2). **(C)** Relative activity of MSRR cis-elements of the AKR1B1 promoter following stimulation with IL-1β of transfected stromal endometrial cells.

CELL TREATMENTS

HIESC-2 (passages 15–22) and HIEEC-22 (passages 15–22) were cultured in RPMI 1640 without phenol red, containing 50 IU penicillin-streptomycin supplemented with 10% whole FBS during growth and 10% dextran-coated charcoal extracted FBS once cells have reached confluence and for transfection or treatments. Knockdown transfection of cells with AKR1B1 specific siRNA was performed with lipofectamine 2000 for 4 h in culture medium without antibiotic. Unless specified, otherwise cells were treated for 24 h with IL-1β (1 ng/ml) or other agents at specified concentrations in RPMI 1640 medium without serum. For Western blot analysis (described above), the cells were grown in 24-well plates

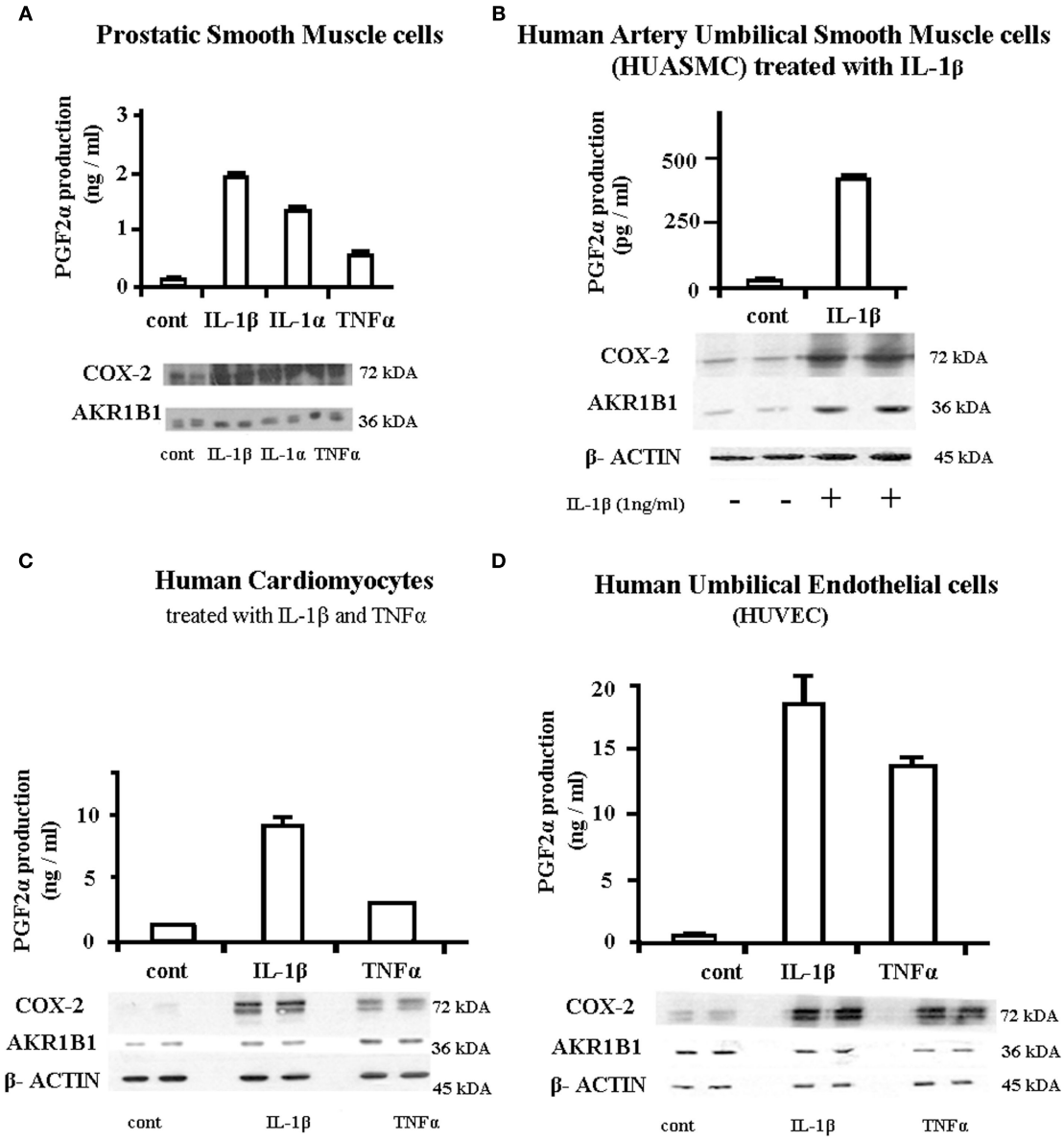

FIGURE 5 | Association between induction of AKR1B1 and PGF2α production following cytokine stimulations in cells from different human tissues. Cells from different human tissues were grown to confluence and treated with IL-1β or IL-1α 1 ng/ml or TNFα at 10 ng/ml for 24 h. The culture medium was recovered for assay of PGF2α by EIA and protein from attached cells recovered for Western blot analysis of COX-2 and AKR1B1. **(A)** Prostatic smooth muscle cells, **(B)** human artery umbilical smooth muscle cells (HUASMC), **(C)** human cardiomyocytes, and **(D)** Human Umbilical Endothelial cells (HUVEC).

whereas for Northern blots analysis (described above), cells were in 6-well plates. At the end of the treatment period, the culture medium was recovered and stored at −20°C until evaluation of PGF2α or PGE2 production by EIA whereas protein and RNA were extracted directly from cell monolayer at the bottom of 24-well and 6-well plates, respectively.

ENDOMETRIAL EXPLANTS CULTURE

Endometrial tissue was obtained either from biopsies collected with an endometrial curette (Pipelle) and obtained from women aged between 25 and 50 years. Informed consent for donation of anonymous endometrial samples was obtained before tissue collection. The research protocol was approved by the Ethics Committee on Human Research of Centre Hospitalier Universitaire de Québec. Endometrial explants were prepared from fresh biopsies as described previously (Bresson et al., 2011). Endometrial explants were stimulated or not with IL-1β in the presence or absence of the aldose reductase inhibitor ponalrestat (100 μM) under normal (5 mM) or high glucose (25 mM) for 24 h.

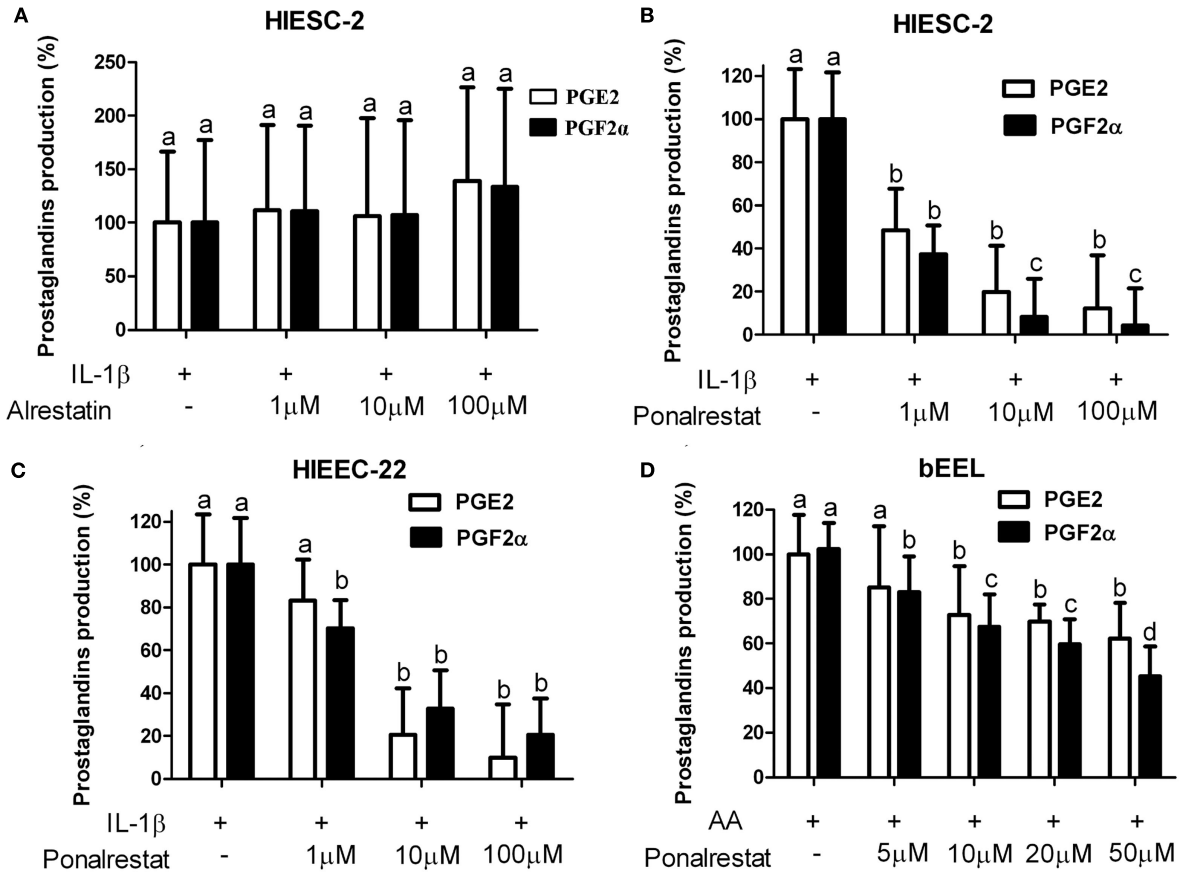

FIGURE 6 | Influence of different aldose reductase inhibitors on PGF2α and PGE2 production in human and bovine endometrial cells. Human endometrial stromal [HIESC-2 **(A,B)**] and human [HIEEC-22 **(C)**] or bovine epithelial [bEEL **(D)**] cells were grown to confluence and treated with 1 ng/ml IL-1β **(A–C)** or 10 μM arachidonic acid (AA, **D**) for 24 h in presence and absence of increasing concentrations of indicated aldose reductase inhibitors. The culture medium was recovered and PGE2 and PGF2α were assayed by EIA. **(A)** Alrestatin **(B–D)** ponalrestat. Results are the mean ± SEM of three experiments run in triplicate.

MEASUREMENT OF SORBITOL

We have developed a LC/MS/MS procedure for quantitative measurement of sorbitol generated in briefly, the culture medium was removed for estimation of PGE2 and PGF2α and sorbitol was extracted from explants by mixing with lysis buffer (200 μl), methanol (600 μl), chloroform (200 μl), and water (500 μl). The mixture was centrifuged for 5 min at room temperature and 100 μl of the upper phase was evaporated with a turbo-vap at 45°C for 30 min. The pellet was resuspended with 200 μl of Pyridine 10%: anhydride acetic acid 10% in acetonitrile and brought at 70°C for 45 min. After drying samples were diluted in 100 μl methanol/water (1:1) containing 5 mM ammonium acetate. The samples were spiked with Sorbitol-d8 (Omicron Biochemicals Inc., IN, USA) also used for establishment of a standard curve and analyzed on an Applied Biosystems API-5000 LC/MS/MS. The procedure was optimized to minimize interference with sugars and alcohols such as glucose, fructose, galactose, myoinositol, and galactitol.

PGE2 AND PGF2α EIA

PGE2 and PGF2α were assayed by competitive EIA using acetylcholinesterase-linked PG tracers (Cayman, Ann Arbor, MI, USA) as described previously (Asselin et al., 1996).

STATISTICAL ANALYSIS

Data were analyzed by One or Two way ANOVA with Bonferroni as *post hoc* test with 95% confidence intervals using GraphPad Prism 5 program. For data with less than three columns a two-tailed unpaired *t* test with 95% confidence intervals was performed using GraphPad Prism 5 program. Data are presented as the mean ± SEM. Each experiment was repeated three times unless specified otherwise. Values were considered statistically significant for $P < 0.05$.

RESULTS

While glucose and oxidized lipids are passive stable substrates for AKR1B1, generation of PGF2α requires the generation of the unstable PGH2 precursor within the producing cell (**Figure 1**). **Figure 2** illustrates that upon stimulation by IL-1β, the rate limiting enzymes cPLA2 releasing AA from membranes phospholipids, COX-2 generating the PGH2 precursor and AKR1B1 are all increased in both endometrial stromal (A) and epithelial (B) cells. The production of PGF2α is also increased following IL-1β stimulation, a response blocked by the COX-2 selective inhibitor NS-398 (C and D). As was found previously (Pelletier et al., 1999; Bresson et al., 2011), AKR1C3 is present only in epithelial cells

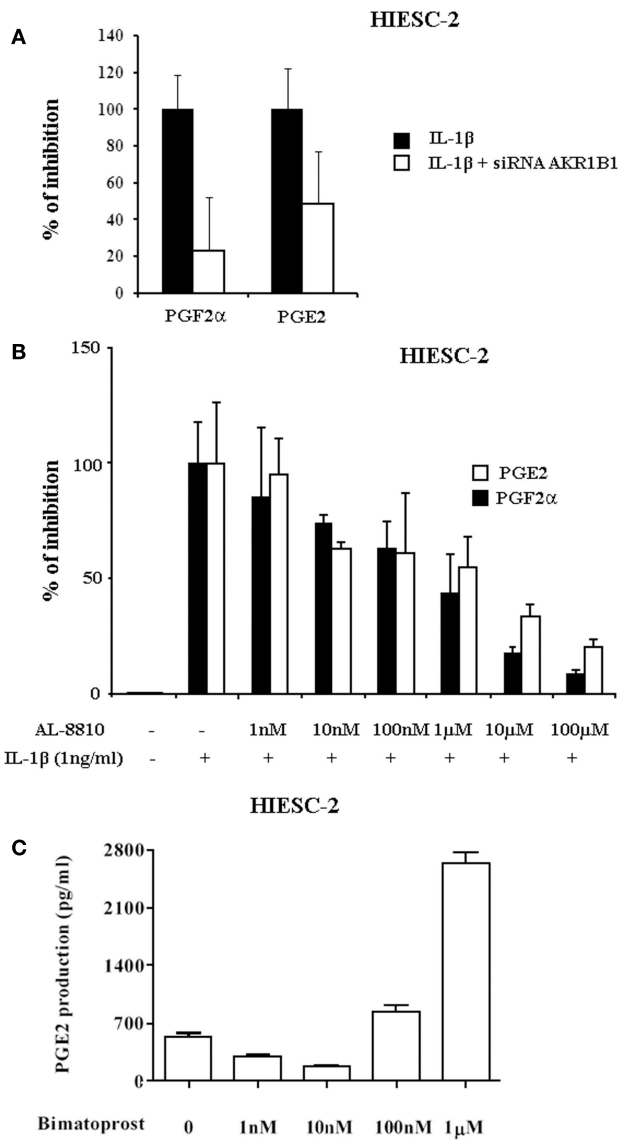

FIGURE 7 | Influence of AKR1B1 and FP receptor inhibition or stimulation on PG production in human endometrial stromal cells. **(A)** Normal or AKR1B1 siRNA transfected human endometrial stromal (HIESC-2) cells were grown to confluence and treated with IL-1β for 24 h and PGE2 and PGF2α were measured in the culture medium. **(B)** HIESC-2 cells were grown to confluence and treated with IL-1β for 24 h in presence and absence of increasing concentrations of the FP receptor antagonist AL-8810, furthermore PGE2 and PGF2α were measured in the culture medium. **(C)** HIESC-2 cells were grown to confluence and treated for 24 h with increasing doses of the PGF2α analog and FP receptor agonist bimatoprost and PGE2 was measured in the culture medium.

and is not induced by IL-1β suggesting minimal contribution to PGF2α production under these conditions.

The time course of PG biosynthetic enzymes activation following IL-1β stimulation was investigated at the mRNA level and associated with PGF2α release (**Figure 3**). Note that there is basal expression of AKR1B1 gene under non-stimulated conditions and that there is serial transcription of biosynthetic enzyme genes with AKR1B1 preceding COX-2 preceding mPGES-1. Accordingly, PGF2α release precedes that of PGE2 in both stromal and epithelial cells ($P < 0.05$).

We have cloned the AKR1B1 promoter (∼4.5 kb) and generated eight constructs to identify potential response elements sensitive to IL-1β stimulation (**Figure 4**). IL-1β stimulates the AKR1B1 promoter activity from-583up in both endometrial stromal and epithelial cells (**Figure 4A**). A MSRR containing two putative antioxidant response elements (ARE) adjacent to TonE and AP1 previously identified for the mouse AKR1B3 (Nishinaka and Yabe-Nishimura, 2005) was also identified for the human AKR1B1 gene at −1710 to −1064 (**Figure 4B**). The human MSRR region was analyzed in a vector containing a minimal promoter SV40 to identify putative cis-elements of the AKR1B1 gene promoter activity involved in IL-1β response (**Figure 4C**). The AREs ARE1 and ARE2 appear particularly efficient to confer IL-1β response and may explain the quick activation of AKR1B1 and PGF2α production observed in **Figure 3**.

The PGF synthase activity of AKR1B1 and its stimulation by IL-1β and TNFα was investigated in cultured cells isolated from different human tissues (**Figure 5**). In addition to endometrial cells where we made our initial discovery, we have found that IL-1β and to a lesser extent TNFα stimulated AKR1B1, COX-2, and PGF2α production in prostatic smooth muscle cells (A), arterial smooth muscle cells (B), cardiomyocytes (C), and umbilical endothelial cells (HUVEC D). In the last two models, the observed increase in PGF2α production appeared to result from increased COX-2 expression coupled with already high preexisting levels of AKR1B1.

We have compared the inhibition potential of three aldose reductase inhibitors; alrestatin, EBPC (not shown) and ponalrestat on human endometrial cells and tested the latter on bovine endometrial epithelial cells (**Figure 6**). Alrestatin did not have any effect on PGF2α production (**Figure 6A**). EBPC inhibited PGF2α production with an apparent EC50 around 10 μM (not shown) whereas ponalrestat was the most potent with an EC50 of 1 μM (**Figure 6B**). Ponalrestat also inhibited PGF2α in human (**Figure 6C**) and bovine (**Figure 6D**) endometrial epithelial cells albeit with reduced efficiency exhibiting an EC50 of 50 μM. Interestingly, ARIs not only inhibited PGF2α but also PGE2 in all cell models.

The potential non-selective inhibition of several components of PG biosynthesis by ARIs was investigated further (**Figure 7**). When AKR1B1 expression was knocked down by siRNA, similar observation of concurrent inhibition of PGF2α and PGE2 was observed (**Figure 7A**) suggesting potential interactions between the two biosynthetic pathways. The possibility that AKR1B1 could exert positive regulation of PGE2 production through PGF2α and its FP receptor was tested (**Figure 7B**). Interestingly, when AKR1B1 and PGF2α production are stimulated with IL-1β, PGF2α, and the concomitant increase of PGE2 is reduced in presence of the FP receptor antagonist AL-8810 suggesting that PGF2α was able to stimulate its own and PGE2 production. The latter hypothesis was tested using the PGF2α analog bimatoprost which stimulated PGE2 release in a dose dependent manner (**Figure 7C**).

Finally, the relative affinity and potential competition between the PGFS and polyol pathway was tested under close to *in vivo*

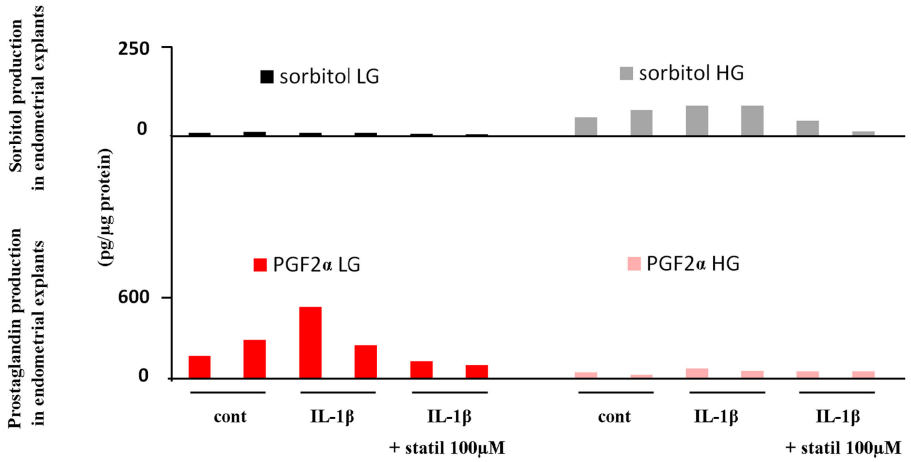

FIGURE 8 | Influence of glucose concentration on the relative production of sorbitol and PGF2α in fresh explants from human endometrium. Endometrial explants were prepared from fresh endometrial biopsies, cultured for 24 h, and stimulated with IL-1β (10 ng/ml) in presence or absence of Statil (ponalrestat, 100 µM) under normal (LG) 5 mg/ml or High (HG) 25 mg/ml glucose concentrations. PGF2α production was measured by EIA. Results from two different experiments are illustrated.

conditions (**Figure 8**). Fresh endometrial explants were treated with IL-1β in presence and absence of ponalrestat under normal and high glucose conditions. Interestingly, IL-1β previously demonstrated to increase AKR1B1 expression increased PGF2α or sorbitol production depending on normal or high glucose conditions respectively. This demonstrates that PGF2α and sorbitol are formed competitively depending on the relative availability of AKR1B1 substrates.

DISCUSSION

Prostaglandins are important regulators of female reproductive function and contribute to gynecological disorders. Normal menstruation depend on an equilibrium between vasoconstrictors such as PGF2α (Lockwood and Schatz, 1996; Sales and Jabbour, 2003a) and vasodilators such as PGE2 or nitric oxide (NO) (Tschugguel et al., 1999). Excessive production of contracting prostaglandins create an ischemia-reperfusion response causing painful menstruation or dysmenorrhea (Okazaki et al., 2005) whereas increased vasodilatation leads to abundant menstrual bleeding (Sales and Jabbour, 2003b). NSAIDs represent the most important and widely used drugs on the market and they are all efficient to treat menstrual disorders at some level. However these drugs act at an early step of biosynthesis common to all PGs and not only the isotype responsible for the pathological response (**Figure 9**). Because of its notorious role on inflammation and pain, the biosynthetic pathway leading to PGE2 has been studied extensively, but that of PGF2α is poorly documented. The data presented in this manuscript address the conditions regulating the newly identified PGFS activity of AKR1B1. In the human endometrium, it has been reported that production of PGF2α is higher in late secretory and menstrual periods of the menstrual cycle (Downie et al., 1974). Accordingly, both AKR1B1 and AKR1C3 enzymes are present in the endometrium throughout the menstrual cycle. By contrast with AKR1B1expressed in both stromal and glandular epithelial cells and modulated in association with endometrial PGF2α production, AKR1C3 expression is constant and completely absent in stromal cells as was reported previously (Pelletier et al., 1999; Zakharov et al., 2010). The absence of the only currently accepted human PGFS AKR1C3 in stromal cells was surprising because we and others have shown that human endometrial stromal cells produce high levels of PGF2α (Huang et al., 1998; Chapdelaine et al., 2006; Kang et al., 2006). Because of a similar finding in the bovine endometrium and our identification of AKR1B5 as a functional PGFS in that system (Madore et al., 2003), we hypothesized that the human AKR1B1, could also express PGFS activity in the human endometrium. Our initial studies showing the association between AKR1B1 expression and PGF2α production was confirmed unequivocally in the human endometrium using gain and loss of function approaches (Bresson et al., 2011). The PGF synthase activity of AKR1B1 and other members of this family is now acknowledged by leaders in the field of prostaglandin synthases (Nagata et al., 2011; Smith et al., 2011; Watanabe, 2011).

In the present study we highlight the necessary co-activation of genes of the PG biosynthesis pathway to provide the PGH2 precursor substrate to AKR1B1 in order to generate PGF2α. This is in clear contrast with detoxification or aldose reductase activities of AKR1B1 for which substrates are provided by extracellular sources (glucose) or as byproducts of intracellular reactions (oxidized lipids). Endometrial cells express constitutively GLUT2 and GLUT3 and do not require insulin for glucose entry, as such; the endometrium belongs to the category of tissues most adversely affected by poorly controlled glucose levels occurring during insulin resistance or diabetes. **Figures 2** and **3** illustrate that genes and proteins of the biosynthetic pathway are increased in a time and dose dependent manner to explain increased PGF2α production following IL-1β stimulation.

In **Figure 3** and then in **Figures 6** and **7** we observe that PGE2 production closely follows that of PGF2α under both stimulatory and inhibitory conditions. We first considered these results

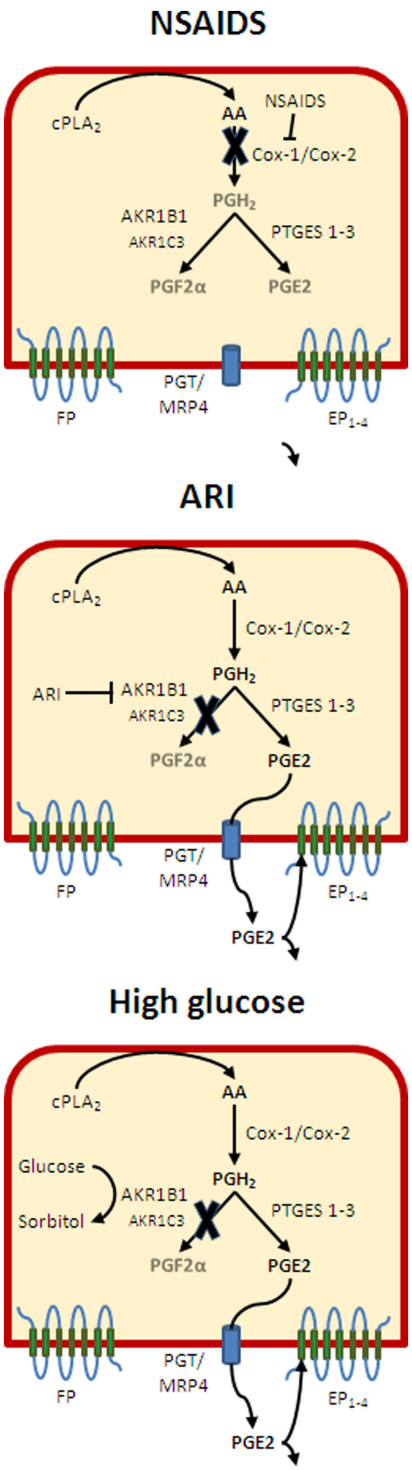

FIGURE 9 | Regulation of PGs of the relative PGE2 and PGF2α release in endometrial cells. Following activation of endometrial cells with the cytokine IL-1β, several enzymes leading to the production of PGF2α and PGE2 exhibit increased expression and/or activity. Treatment of cells with NSAIDs will inhibit the generation of PGH2 at the level of COX-1 and COX-2, thus blocking the release of both PGF2α and PGE2 without an alteration of the relative production of the two PGs. Inhibition of AKR1B1 *(Continued)*

FIGURE 9 | Continued
activity with ARIs will inhibit preferentially PGF2α release thus creating an imbalance in favor of PGE2. However, decreased stimulation of the FP receptor should down-regulate the production of PGE2. In presence of high glucose concentration, the stimulation of AKR1B1 may be exacerbated, but glucose should inhibit competitively PGF2α release in which case reduced FP stimulation should also reduce PGE2 production. However, when glucose concentration falls back to normal, high AKR1B1 levels would favor PGF2α release and ischemic responses until PGE2 is able to compensate through EP2 and bring back a silent condition where the balance between PGF2α and PGE2 is re-established.

as artifact and resulting from defective experimental design. However, when we observed that inhibition of FP receptor prevented the increase in PGE2 and that FP receptor stimulation alone was sufficient to stimulate PGE2 production, we proposed that there was a positive feedback loop between PGF2α release and production of PGE2. A link between FP activation and increased PG production could have been anticipated from previous observations in a human endometrial model (Jabbour et al., 2005; Sales et al., 2008) and from our own observations in bovine endometrial cells (Krishnaswamy et al., 2010) given that FP and the oxytocin receptor (OT) share the same signal transduction systems. However, this is the first evidence of a specific link between FP activation and PGE2 production within a homogeneous cell system with potential physiological and pathological relevance. If we hypothesize that PGE2 acting on EP2 receptors and PGF2α acting through FP work as a dyad with opposite actions in the reproductive system, just like the thromboxane/prostacyclin dyad in the vascular system, then the interactions between the two systems are not only probable but also necessary.

Prostaglandins work like a micro endocrine system sensing altered conditions and reestablish locally, optimal conditions for tightly regulated events such as ovulation or recognition of pregnancy in the reproductive system or hemostasis in the vascular system. In this respect, PGF2α exhibits vasoconstrictive and prothrombotic responses comparable to thromboxane with the additional feature that the PGF2α molecule is chemically stable and remains active until catabolism by prostaglandin dehydrogenase, usually through a single passage in the lung. By contrast PGE2/EP2 trigger a vasodilator response with anti thrombotic action similar to prostacyclin but again PGE2 is much more stable than PGI2. Thus it is important to tightly regulate the relative production and release of PGF2α and PGE2 and in this respect; our identification of a feedback loop between PGF2α and PGE2 within endometrial cells has important implications for the observed complications associated with over-expression of AKR1B1. We have initiated the characterization of the AKR1B1 promoter and identified a MSRR containing two putative AREs adjacent to TonE and AP1. The AREs ARE1 and ARE2 appear to be involved in IL-1β response, which supports the local sensing functions of PGs to which AKR1B1 is now a contributing component. The MSRR portion of the AKR1B1 promoter will have to be investigated further under IL-1β stimulation to identify the trans-factors associated with the production of PGF2α. Because of its reported interaction with AREs, NRF2 also known as Nuclear factor (erythroid-derived 2)-like 2 (NFE2L2) is a likely candidate to link increased PGF2α release

under inflammatory conditions (Nishinaka and Yabe-Nishimura, 2005).

Clinical and pathological implications: Prostaglandins are important regulators of female reproductive functions. In this respect, gene inactivation studies in the mouse have shown that COX-2 and EP2 null mice share similar phenotypes where ovulation and implantation are impaired (Lim et al., 1997; Kennedy et al., 1999). The PGF2α/FP system is much less documented than that of PGE2, but the group of Jabbour in Edinburg has documented its involvement in endometrial pathologies including menstrual disorders and endometrial cancer (Milne and Jabbour, 2003; Jabbour and Sales, 2004; Sales et al., 2004; Jabbour et al., 2005, 2006; Abera et al., 2010; Catalano et al., 2011) while we (Breuiller-Fouche et al., 2010; Phillips et al., 2011) and others (Olson and Ammann, 2007) associate it with premature delivery. With that in mind, it is likely that AKR1B1 contribute to these pathologies and conversely conditions where AKR1B1 is affected such as diabetes and metabolic syndrome may exhibit a specific prognosis pattern in affected women. In addition, we claim that the female reproductive system constitutes a powerful model to understand the contribution of PGs to physio-pathological conditions in other systems. This is supported by **Figure 5** showing the relation between AKR1B1 and PG biosynthetic enzymes in response to IL-1β and their association with PGF2α production in smooth muscles, cardiomyocytes, and endothelial cells. This is all the more important as AKR1B1 known to be highly responsive to diverse physiologic and pathologic conditions and its multiple substrate processing ability combine to create complex combinations well illustrated in **Figure 8**. For instance, PGF2α has been associated with cardiac hypertrophy (Lai et al., 1996) and a new hamster AKR1B was identified and proposed to contribute to cardiomyopathy through PGF2α release (Sakamoto and Sugamoto, 2011). Moreover, knowing that high glucose induce AKR1B1 expression we see that as long as glucose levels remain high, there will be little increase in PGF2α release, but when glucose goes down, the high levels of AKR1B1 will generate increased production of PGF2α (**Figure 9**). In turn, high PGF2α will trigger pro-ischemic responses (Yuhki et al., 2010) that fortunately will be compensated by the feedback loop release of PGE2 exerting opposite action thus bringing back homeostasis (Mandal et al., 2005). Normal tension results from the action of EP2 opposing FP receptors in the vascular system, but adjacent tissues also express other EP receptors responsible for inflammation and pain. Therefore the relative silent condition resulting from PGE2 compensation of overproduction of PGF2α by AKR1B1 will likely generate pain or hyperalgesia increasing the probability to take NSAIDs. Not that far ago, the best pain killer on the market with minimal gastrointestinal toxicity was rofecoxib (Vioxx), the most powerful medication to date eradicating pain by reducing mPGES-1 generated PGE2. If someone taking Vioxx was under the silent (increased AKR1B1) condition, then the compensatory PGE2/EP2 would not be available to counteract the ischemic response to PGF2α thus increasing the risks of heart failure. Interestingly, even after adjustment of risk factors, reexamination of Vioxx events (Baron et al., 2008) reveal that people suffering from type two diabetes were eight times more at risk to die from Vioxx than non-diabetics. By contrast people taking both Vioxx and Aspirin, thus blocking the AKR1B1 generated PGF2α were protected. Therefore, it is worth investigating if and how AKR1B1 potentially contributed to the failure of Vioxx.

In conclusion, the ability of AKR1B1 to release PGF2α brings a new angle to look at mechanisms responsible for pathophysiologic conditions in the woman reproductive and other systems especially in association with diabetes.

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Expression of the aldo-ketoreductases AKR1B1 and AKR1B10 in human cancers

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The American Cancer Society estimates that there will be more than 1.5 million new cases of cancer in 2011, underscoring the need for identification of new therapeutic targets and development of novel cancer therapies. Previous studies have implicated the human aldo-ketoreductases AKR1B1 and AKR1B10 in cancer, and therefore we examined *AKR1B1* and *AKR1B10* expression across all major human cancer types using the Oncomine cancer gene expression database (Compendia Biosciences, www.oncomine.com). Using this database, we found that expression of *AKR1B1* and *AKR1B10* varies greatly by cancer type and tissue of origin, including agreement with previous reports that *AKR1B10* is significantly over-expressed in cancers of the lungs and liver. *AKR1B1* is more broadly over-expressed in human cancers than *AKR1B10*, albeit at a generally lower magnitude. *AKR1B1* over-expression was found to be associated with shortened patient survival in acute myelogenous leukemias and multiple myelomas. High *AKR1B10* expression tends to predict less aggressive clinical course generally, notably within lung cancers, where it tends to be highly over-expressed compared to normal tissue. These findings suggest that AKR1B1 inhibitors in particular hold great potential as novel cancer therapeutics.

Keywords: AKR1B1, AKR1B10, HSIR, aldose reductase, cancer, leukemia, meta-analysis

INTRODUCTION

Cancer is the second leading cause of death in the U.S. behind heart disease, and the American Cancer Society estimates that there will be more than 1.5 million new cases of cancer in 2011. While improvements in detection, treatment, and prevention have led to decreases in cancer deaths and incidence for many cancer types in the U.S., the incidence rate for some cancers such as hepatocellular carcinoma is still rising. However, as the U.S. population ages, cancer incidence may reach a plateau or even rebound. Therefore, identification of new therapeutic targets and development of novel cancer therapies is still a pressing need. Previous studies have shown that the human aldo-ketoreductase AKR1B10 is over-expressed in cancers of the liver and lungs (Fukumoto et al., 2005; Woenckhaus et al., 2006; Heringlake et al., 2010; Kang et al., 2011; Schmitz et al., 2011) while AKR1B10 and the related enzyme AKR1B1 are both linked to drug resistance in cancer-derived cell lines (Dan et al., 2003; Plebuch et al., 2007; Matsunaga et al., 2011; Zhong et al., 2011). Several published studies have also pointed to role for AKR1B1 in colon carcinogenesis (Tammali et al., 2009, 2011a,b; Ramana et al., 2010). As well-characterized inhibitors for these enzymes are already in use or development, they would seem to be attractive targets for cancer therapeutic development. However, these studies have been conducted largely in model systems, and thus there is very little known about the involvement of AKR1B10 in cancer outside of the lungs and liver, and while *AKR1B1* expression has been reported to be elevated in human cancers, this study was limited by a small number of available patient samples (Saraswat et al., 2006). Since AKR1B1 has been shown to be involved in many cellular processes relevant to cancer such as EMT (Zablocki et al., 2011), inflammation (Yadav et al., 2007, 2009, 2011), and angiogenesis (Tammali et al., 2011b,c), and AKR1B10 is known to have relevance to human cancers, we examined *AKR1B1* and *AKR1B10* expression across all major human cancer types using the Oncomine cancer gene expression database (Compendia Biosciences, www.oncomine.com).

Using this database, we found that expression of *AKR1B1* and *AKR1B10* varies greatly by cancer type and tissue of origin, including agreement with previous reports that AKR1B10 is significantly over-expressed in cancers of the lungs and liver (Fukumoto et al., 2005; Woenckhaus et al., 2006; Heringlake et al., 2010; Kang et al., 2011; Schmitz et al., 2011). While under-expression of AKRs in human cancers is less common than over-expression, *AKR1B1* appears to be generally under-expressed in prostate cancers compared to normal tissue while *AKR1B10* expression is reduced in colon tumors. *AKR1B1* over-expression was associated with shortened patient survival in acute myelogenous leukemias and multiple myelomas. High *AKR1B10* expression tends to predict less aggressive clinical course generally, notably within lung cancers, where it tends to be highly over-expressed compared to normal tissue. Neither *AKR1B1* nor *AKR1B10* appears to have notable associations with disease recurrence, and their associations with the presence of metastases are inconsistent.

These findings suggest that AKR1B1 in particular may be a promising drug target, due to its broad over-expression in solid tumors and leukemias. Previous drug development attempts centered on AKR1B1 inhibition in non-cancer disease states were halted due to unacceptable toxicity; however, the reported toxicities were milder than other chemotherapeutics currently in use. Newer AKR1B1 inhibitors such as those derived from natural products (Suryanarayana et al., 2004, 2007; Saraswat et al.,

2008) may have lower toxicity than earlier compounds, and therefore hold great potential as novel therapeutics for cancers where *AKR1B1* tends to be over-expressed.

MATERIALS AND METHODS

Meta-analysis of *AKR1B1* and *AKR1B10* gene expression in human cancers and normal tissues as well as related statistical analysis were conducted using the Oncomine gene expression database (www.oncomine.com, Compendia biosciences, Ann Arbor, MI, USA). Where appropriate, raw data was downloaded from Oncomine and scrutinized to ensure consistent comparisons and definitions such as "high grade" were used across different studies. For example, in prostate tumors "high gleason score" is defined by the highest grade tumors within the study being considered, which for some studies is GS7 and in others GS10. *P*-values were determined by Student's *t*-test and those less than 0.05 were considered significant. Gene rank represents the ordered numerical rank of that gene's *p*-value against all other genes for that comparison – i.e., a gene with a rank of 5 has a more significant difference in expression level for the two conditions examined than for all but four other genes. In data presented considering over-expression versus under-expression in a given cancer type, the most significant *p*-value and gene rank are presented even in cases where neither were significant as defined by $p < 0.05$. Only studies based on human clinical samples were included in our analyses. Where an "overall *p*-value" is listed, the *p*-value generated by simultaneously considering all available data within Oncomine for the given comparison is displayed, i.e., the overall *p*-value for the cancer versus normal comparisons listed in **Table 1** includes all studies for which gene expression for cancerous and corresponding normal tissue was available within Oncomine. Median gene ranks displayed are similarly inclusive of all available data in Oncomine. All graphics displayed in figures are Oncomine.svg file output modified by Adobe Illustrator.

RESULTS

To determine whether the aldo-ketoreductases *AKR1B1* and *AKR1B10* were differentially expressed between cancerous and normal tissues, we broadly examined microarray data from patient samples contained within the Oncomine database. Results from cancer types where a statistically significant difference in AKR expression between the cancerous and corresponding normal tissue exists are summarized in **Table 1**. The cancers where gene expression for AKRs was compared to the corresponding normal tissue, but no overall significant difference was found were certain brain tumors (oligodendrogliomas, mixed gliomas), ductal and lobular breast cancers, acute myeloid leukemias, myelomas, and ovarian cancers (not shown). Data for cancers where only one study was available for analysis is also not shown. *AKR1B1* expression is significantly elevated compared to the corresponding normal tissue in bladder, brain (astrocytomas and glioblastomas), cervical, esophageal, head and neck, kidney, leukemias (T-cell acute, B-cell acute, and chronic), lymphomas, and melanomas (**Table 1**; **Figure 1A**). The fold change in gene expression versus the normal tissue is summarized by study in **Table 1**, with *AKR1B1* expression ranging from ∼1.2- to 5-fold the normal tissue in the majority of cancers where it is significantly over-expressed. The most significant differences between *AKR1B1* expression in cancerous and normal tissue are seen in leukemias (**Table 1**; **Figure 1A**). *AKR1B1* expression is significantly lower than the corresponding normal tissue in prostate cancers (**Table 1**). As previously reported, *AKR1B10* is over-expressed in liver and lung tumors (**Table 1**; Fukumoto et al., 2005; Woenckhaus et al., 2006; Heringlake et al., 2010; Kang et al., 2011; Schmitz et al., 2011), with fold change relative to normal tissue ranging from 12- to 67-fold in liver cancers; 2- to 75-fold in squamous cell lung cancers; and 1.5- to 5.5-fold in lung adenocarcinomas (**Table 1**). *AKR1B10* is also significantly over-expressed in leukemias (T-cell acute, B-cell acute, and chronic) and pancreatic cancers (**Table 1**; **Figure 1A**). *AKR1B10* over-expression thus appears to be less common than *AKR1B1* over-expression in cancer, and *AKR1B10* is under-expressed in colon, gastric, and head and neck cancers (**Table 1**). It should be noted that these associations are those that hold true across the studies contained within Oncomine, and multiple studies may have individually held a significant association of AKR expression with either the cancerous or normal state, but not in the broader comparison. Our methods also necessarily exclude studies not contained within the Oncomine database.

As shown in **Figure 1**, even for leukemia types in which AKRs are over-expressed compared to normal tissue at a high level of statistical significance, there is considerable heterogeneity amongst patients in terms of *AKR1B1* and *AKR1B10* expression (**Figure 1A**). This led us to ask whether AKR expression could identify certain types of patients within these leukemias, and we found that high levels of *AKR1B1* expression within B-cell leukemia patients was strongly associated with the presence of the TCF3-PBX1 gene fusion (**Figure 1B**), while under-expression of *AKR1B1* in chronic myelogenous leukemias was associated with the presence of the PML-RARA gene fusion (**Figure 1C**). Across all translocations and gene fusions in all leukemia types, *AKR1B1* over-expression is associated with the TCF3-PBX1 gene fusion and 11q23 MLL rearrangements, while under-expression is associated with the PML-RARA and ETV6-RUNX1 gene fusions (**Figure 2**). Other gene fusions, translocations, and point mutations examined in leukemias did not have a statistically significant, consistent pattern (**Figure 2** and data not shown).

We next asked whether expression of AKRs might be able to predict clinical outcome, specifically in terms of patient survival, disease recurrence, tumor grade, and metastasis. We found no significant associations with expression of *AKR1B1* and the presence of metastasis, tumor grade, or with disease recurrence in any cancer type, though some individual studies sometimes contained a significant relationship that did not hold up when all available data for that cancer type was considered (data not shown). *AKR1B1* over-expression was associated with decreased patient survival at 1 year post-prognosis in acute myeloid leukemias (**Figure 3A**), as well as decreased patient survival at 1 year post-prognosis in multiple myeloma (**Figure 3B**). *AKR1B1* over-expression was also associated with decreased survival in pancreatic cancer, however, only one small study (27 patients) within Oncomine contained patient survival data (data not shown). While no significant associations of patient survival with *AKR1B10* expression were observed, it is noteworthy that in the solid tumors where *AKR1B10* is most highly over-expressed, namely liver cancer and squamous cell lung carcinoma, there is a strong trend for *AKR1B10* over-expression predicting longer patient survival (**Figure 3C**).

Table 1 | AKR expression in human cancers.

Gene	Cancer type	Mean fold change(s) versus normal tissue, by study	Overall *p*-value	Median gene rank
AKR1B1	Bladder (infiltrating)	1.77, 1.26, 1.09	0.007	3659
AKR1B10	Bladder (infiltrating)	1.18, −1.80, −2.61, −3.72	0.107	2596
AKR1B1	Brain (astrocytomas)	2.31, 1.96, 1.96, 1.39, 1.27, 1.22	0.002	374.5
AKR1B10	Brain (astrocytomas)	1.12, −1.28, −1.27, −1.27, −1.09	0.074	5854
AKR1B1	Brain (glioblastomas)	2.15, 1.31, 1.23, 1.21, −1.64	0.002	5624
AKR1B10	Brain (glioblastomas)	1.46, 1.07, 1.02, −1.18, −1.00	0.421	7509
AKR1B1	Cervical	2.90, 2.12, 2.09, 1.14	1.32E-06	1152.5
AKR1B10	Cervical	1.09, −6.97, −5.30, −1.10	0.256	5532
AKR1B1	Colon	1.07, −1.50, −1.28, −1.12, −1.12, −1.06, −1.05, −1.04	0.118	4914
AKR1B10	Colon	−30.67, −15.31, −12.80, −10.60, −7.25, −1.47, −1.41	2.97E-09	252
AKR1B1	Esophageal	4.52, 2.99, 1.88, 1.54, 1.29	9.19E-04	3294
AKR1B10	Esophageal	1.68, 1.09, -3.17, −2.22, −2.02, −1.79	0.118	4784.5
AKR1B1	Gastric	1.18, 1.06, 1.04	0.116	8244
AKR1B10	Gastric	−8.15, −4.61	0.001	469
AKR1B1	Head and neck	2.61, 1.77, 1.76, 1.41	4.44E-04	619.5
AKR1B10	Head and neck	−5.01, −2.19, −2.07, −1.10	0.043	2276.5
AKR1B1	Kidney	3.11, 3.00, 2.85, 2.59, 2.42, 2.01, 1.90	3.61E-05	938.5
AKR1B10	Kidney	5.11, 1.73, 1.54, 1.01, −1.71, −1.35 −1.09	0.257	7125.5
AKR1B1	Leukemia (B-cell acute)	5.32, 2.39, 2.31, 1.92	6.95E-36	600
AKR1B10	Leukemia (B-cell acute)	3.38, 1.11, 1.08, 1.06	8.30E-06	5014.5
AKR1B1	Leukemia (T-cell acute)	4.52, 1.52, -1.02	1.91E-13	2762
AKR1B10	Leukemia (T-cell acute)	3.09, 1.05, −1.21	0.006	7632
AKR1B1	Leukemia (chronic)	1.27, 1.26, −1.48	1.47E-09	4993
AKR1B10	Leukemia (chronic)	1.07, −2.60, −1.23	6.02E-05	6924
AKR1B1	Liver	2.19, 1.28, 1.22, 1.09	0.066	5007.5
AKR1B10	Liver	66.99, 20.82, 14.49, 12.68	1.75E-11	366
AKR1B1	Lung (adenocarcinoma)	1.02, -2.43, −1.25, −1.19, −1.10, −1.08	0.374	4551
AKR1B10	Lung (adenocarcinoma)	5.62, 3.28, 2.58, 1.92, 1.57	4.90E-04	3068
AKR1B1	Lung (squamous)	1.42, -1.24, −1.11, −1.06	0.593	5655.5
AKR1B10	Lung (squamous)	74.71, 66.92, 34.11, 2.03	0.001	483
AKR1B1	Lymphoma	1.86, 1.33, 1.26	0.016	2006
AKR1B10	Lymphoma	1.08, −1.49, −1.22	0.116	5095
AKR1B1	Melanoma	2.43, 1.74, 1.03	0.006	1394
AKR1B10	Melanoma	1.01, 1.01, −1.48	0.266	8928
AKR1B1	Pancreatic	1.74, 1.74, 1.43, 1.41, 1.35, 1.33, 1.17, −2.47	0.069	2479.5
AKR1B10	Pancreatic	13.62, 5.32, 3.21, 2.91, 1.95, −2.56, −1.59	0.003	3600
AKR1B1	Prostate	−1.75, −1.71, −1.58, −1.53, −1.52, −1.48, −1.48, −1.45, −1.41, −1.37, −1.35, −1.31, −1.25, −1.17	0.01	667.5
AKR1B10	Prostate	1.61, 1.3, 1.16, 1.14, 1.05, 1.01, 1.00, -2.20, −1.55, −1.38, −1.03	0.878	5846

Expression of AKR1B1 and AKR1B10 mRNA was examined in all tumor types and hematological malignancies contained within the Oncomine database. Displayed in this table are the average fold changes for each study analyzed, overall p-value, and median gene rank for all cancer types where the overall p-value was significant for either under-expression (blue) or over-expression (red) of either AKR gene examined.

DISCUSSION

In this report we show that *AKR1B1* and *AKR1B10* are over-expressed, and less frequently under-expressed, in a cancer-type-specific manner. *AKR1B10* is most prominently up-regulated in cancers of the liver and lungs, consistent with previous reports (Fukumoto et al., 2005; Woenckhaus et al., 2006; Heringlake et al., 2010; Kang et al., 2011; Schmitz et al., 2011). *AKR1B1* over-expression is more common amongst different tumor types than *AKR1B10* over-expression, but at a generally lower magnitude (**Table 1**). Under-expression is less common for either AKR, with *AKR1B1* under-expressed in prostate tumors and *AKR1B10* under-expressed in colon and head and neck cancers (**Table 1**). Increased *AKR1B1* expression is also associated with the TCF3-PBX1 gene fusion and 11q23 MLL rearrangement in acute leukemias, while decreased expression is associated with the PML-RARA and ETV6-RUNX1 gene fusions (**Figure 2**). Only *AKR1B1* expression has a significant association with clinical outcome, being associated with reduced survival in acute myeloid leukemias and multiple myeloma (**Figure 3**). Recent reports have implicated AKRs in cellular responses to various stresses, including promotion of hypoxia-driven HIF1a signaling, inflammation, and resistance to chemotherapeutics (Dan et al., 2003; Plebuch et al.,

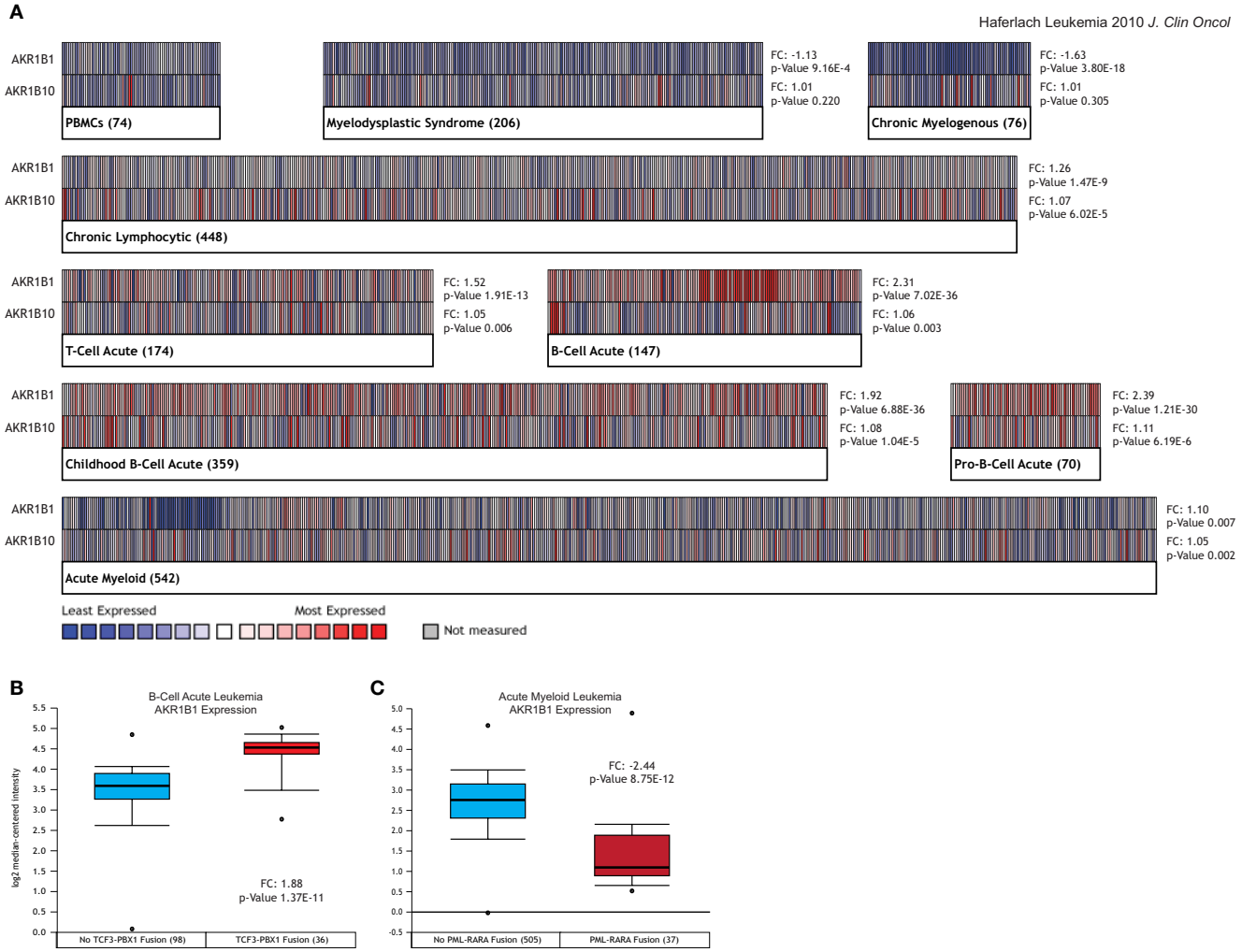

FIGURE 1 | AKR expression in leukemia patients. Expression of AKR1B1 and AKR1B10 mRNA was examined in 2093 human leukemia patients from the Haferlach et al. (2010) study in the Oncomine database. **(A)** Heatmap display of AKR1B1 (top rows) and AKR1B10 (bottom rows) expression within the indicated leukemia types. Fold changes relative to peripheral blood mononuclear cells (PBMCs) and *p*-value for that comparison within each leukemia type are listed next to their respective heatmaps. Numbers in parentheses next to the label of each heatmap represent the number of patients contained within that group in this study. **(B)** AKR1B1 mRNA expression in B-cell acute leukemia patients without (blue box) and with (red box) the presence of the TCF3-PBX1 fusion gene. **(C)** AKR1B1 mRNA expression in acute myeloid leukemia patients without (blue box) and with (red box) the presence of the PML-RARA fusion gene.

2007; Yadav et al., 2007, 2009, 2011; Matsunaga et al., 2011; Zhong et al., 2011). *AKR1B1* over-expression has also been associated with an EMT-like phenotype, is implicated in colon carcinogenesis, and notably, increased AKR1B1 protein expression and enzymatic activity has been reported in several cancer types (Saraswat et al., 2006; Tammali et al., 2009, 2011a,b; Ramana et al., 2010; Zablocki et al., 2011), further suggesting that AKRs play a functional role in tumor growth. Given the broad over-expression of AKRs, particularly *AKR1B1*, in human cancers and the critical processes that they appear to regulate, AKRs have potential to be useful therapeutic targets. AKR inhibitors have been in development for complications related to diabetes for many years, as AKR1B1 and the polyol pathway have been implicated in the pathogenesis of diabetic retinopathy, nephropathy, and cataract (Makiishi et al., 2003; Suryanarayana et al., 2004, 2007; Wolford et al., 2006; Reddy et al., 2008, 2011; Zablocki et al., 2011). While many of these AKR inhibitor drug development efforts have been halted due to toxicity, they exhibit much lower toxicity than many current cancer therapies.

AKR1B1 expression is increased by high blood glucose via NF-κB (Yang et al., 2008), providing a potential mechanism by which diabetes and elevated risk of developing certain cancers may be linked. AKR1B1 and the polyol pathway also contribute to hyperglycemic pseudohypoxia, which one could imagine linking the Warburg effect to tumor angiogenesis through HIF1a and perhaps bolstering neovascularization at oxygen tensions that would not normally promote it. Consistent with this, VEGF has been linked to diabetic retinopathy and nephropathy (Aiello et al.,

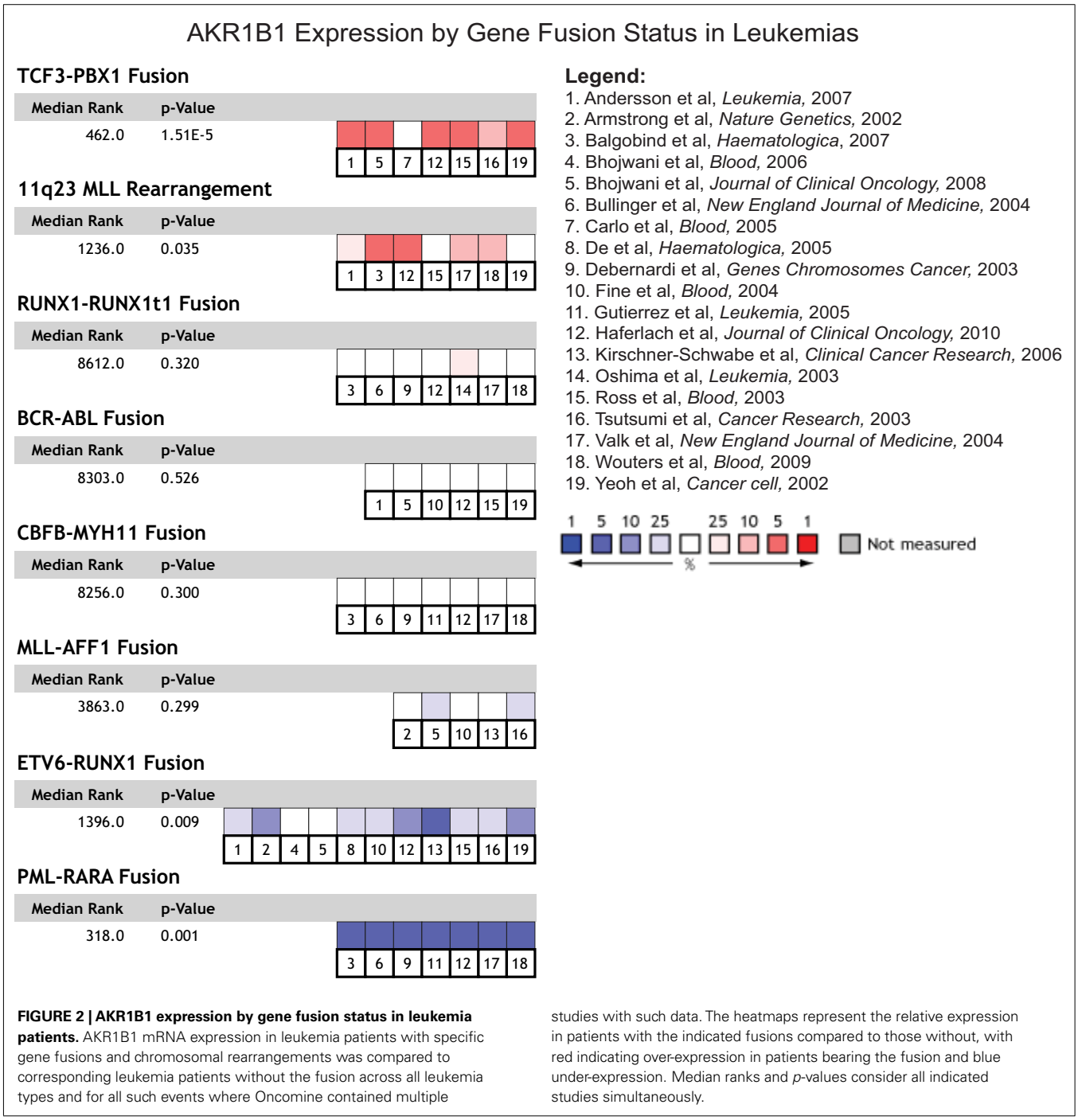

FIGURE 2 | AKR1B1 expression by gene fusion status in leukemia patients. AKR1B1 mRNA expression in leukemia patients with specific gene fusions and chromosomal rearrangements was compared to corresponding leukemia patients without the fusion across all leukemia types and for all such events where Oncomine contained multiple studies with such data. The heatmaps represent the relative expression in patients with the indicated fusions compared to those without, with red indicating over-expression in patients bearing the fusion and blue under-expression. Median ranks and *p*-values consider all indicated studies simultaneously.

1994; Cha et al., 2000; Ozaki et al., 2000), perhaps downstream of AKR1B1-driven pseudohypoxic effects. Intriguingly, patients with Von Hippel–Lindau disease often develop retinal angiomas and kidney tumors, suggesting that VHL-associated malignancies and diabetic complications may differ primarily by the degree of HIF1a and/or VEGF-dysregulation present. It is possible that diabetics are effectively primed to promote tumorigenesis by virtue of an already abnormally high level of hypoxia/HIF1a signaling. In light of all the signs pointing to the involvement of AKRs in human cancers, we hypothesize that AKRs are functionally linked to cancer progression, if not initiation as well. We also propose that AKR inhibitors would have value as cancer therapeutics in cancers that typically feature AKR over-expression, especially in the case of AKR1B1.

REFERENCES FOR STUDIES ANALYZED WITHIN THE ONCOMINE DATABASE

We apologize to our colleagues whose papers used in the meta-analyses are not cited here due the cumbersome nature of including these hundreds of references. For a list of studies used in the analyses for a given tumor type, please contact the authors.

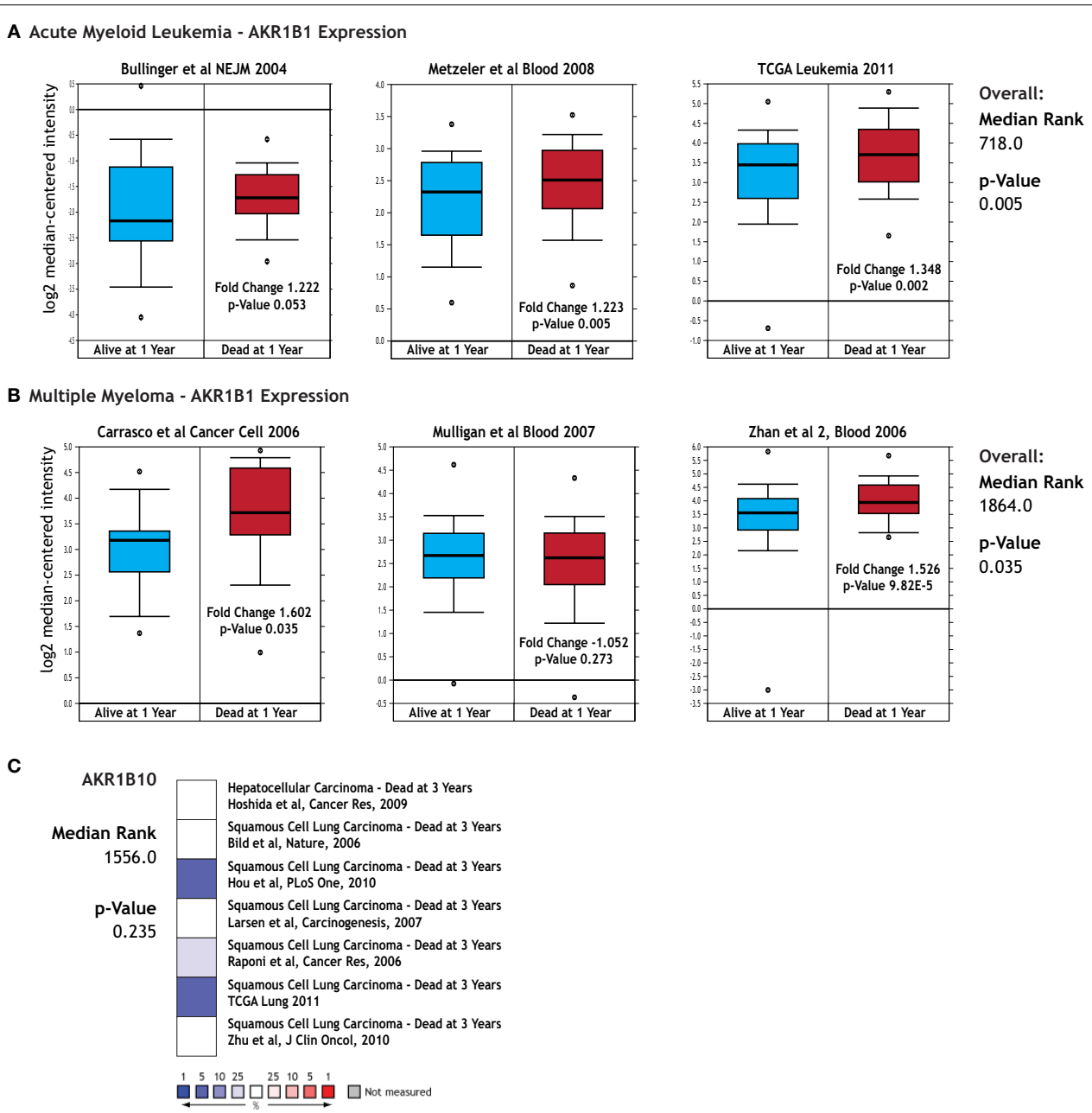

FIGURE 3 | AKR gene expression and clinical outcome. The relationship between AKR1B1 and AKR1B10 mRNA expression and clinical outcomes were examined using the Oncomine database. **(A)** Comparison of AKR1B1 expression in acute myeloid leukemia patients who were alive (blue boxes) and dead (red boxes) at 1 year post diagnosis. Individual *p*-values are indicated within each box plot and the *p*-value and median gene rank for all three studies is at the right of the panel. **(B)** Comparison of AKR1B1 expression in multiple myeloma patients who were alive (blue boxes) and dead (red boxes) at 1 year post diagnosis. Individual *p*-values are indicated within each box plot and the *p*-value and median gene rank for all three studies is at the right of the panel. **(C)** AKR1B10 expression relative to clinical outcome in liver cancer and squamous cell lung cancer. Colored boxes are a heatmap-style representation of AKR1B10 expression in patients dead relative to those alive at 3 years post diagnosis, with blue indicating under-expression and red over-expression. Median rank and *p*-value for this panel considers all indicated studies simultaneously.

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Basal and regulatory promoter studies of the AKR1C3 gene in relation to prostate cancer

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Background: Human 17β-hydroxysteroid dehydrogenase type 5 (17β-HSD5) formally known as aldo-keto reductase 1C3 (AKR1C3) play a major role in the formation and metabolism of androgens. The enzyme is highly expressed in the prostate gland and previous studies indicate that genetic variation in the AKR1C3 gene may influence the prostate volume and risk of prostate cancer. **Aim:** Here we aimed to further study the genetic regulation of AKR1C3 and its putative role in prostate cancer. **Experiments:** A previously identified promoter polymorphism (A>G, rs3763676) localized at −138 from the translational start site were studied in relation to prostate cancer in a Swedish population based case–control study including 176 patients diagnosed with prostate cancer and 161 controls. Moreover, we have studied the basal and androgen induced promoter activity of the AKR1C3 gene. Expression studies with AKR1C3 promoter reporter constructs were performed in HepG2 and DSL2 cells. **Results:** We found that carriers of the promoter A-allele had a borderline significant decreased risk of prostate cancer (OR = 0.59; 95% CI = 0.32–1.08). We also show that dihydrotestosterone (DHT) induced the promoter activity of the A-allele 2.2-fold ($p = 0.048$). Sp3 seem to play an important role in regulating the transcription activity of AKR1C3 and site-directed mutagenesis of a GC-box 78 base-pair upstream the ATG-site significantly inhibited the basal AKR1C3 promoter activity by 70%. **Conclusion:** These results further supports previous findings that the A>G promoter polymorphism may be functional and that AKR1C3 plays a critical role in prostate carcinogenesis. Our findings also show that the members of Sp family of transcription factors are important for the constitutive expression of AKR1C3 gene.

Keywords: AKR1C3, 17BHSD5, prostate cancer, DHT, polymorphism, gene regulation

INTRODUCTION

Human 17β-HSD type 5 belongs to the aldo-keto reductase (AKR) superfamily (Jez et al., 1997) and is formally known as AKR1C3. It is a promiscuous enzyme that participates in the biosynthesis and metabolism of a variety of substrates including androgens, estrogens (Penning et al., 2000), prostaglandins (Matsuura et al., 1998), and polycyclic aromatic hydrocarbons (PAH; Palackal et al., 2002). AKR1C3 is widely expressed in human tissues and is predominant in the prostate and mammary gland (Penning et al., 2000).

The 17-keto reductase activity of AKR1C3 reduces the weak androgen Δ^4-androstenedione (4-dione) to testosterone. The combined 3α/3β activity of the enzyme (Steckelbroeck et al., 2004) partly inactivates 5α-dihydrotestosterone (DHT) to the weak androgens 3α-androstanediol (3α-Adiol) and 3β-androstanediol (3β-Adiol) and the 17β-hydroxysteroid oxidase activity of the enzyme oxidizes 3α-Adiol to androsterone instead of back to DHT (Penning et al., 2000).

The prostate gland is generally considered an important site of DHT formation and inactivation (Thigpen et al., 1993). DHT stimulates the proliferation of the prostate and AKR1C3 has been shown to be up-regulated in localized and advanced prostate adenocarcinoma (Nakamura et al., 2005; Fung et al., 2006; Stanbrough et al., 2006).

Previously an A>G polymorphism in the promoter region (rs3763676) at nucleotide position −138 from the translation start site was identified (Qin et al., 2006). The G-allele has been associated with lower promoter activity in human liver (HepG2), lung (A549), and prostate (LNCaP) cells (Jakobsson et al., 2007) and higher promoter activity in rat theca cells (Qin et al., 2006). The allele frequency of this SNP was shown to be significantly increased in patients with polycystic ovary syndrome (PCOS; Qin et al., 2006) and increased risk for bladder cancer (Figueroa et al., 2008) whereas its involvement in prostate cancer has not been studied.

Previous studies of the AKR1C3 promoter have shown that a 169-bp region (−104 to +65) is capable of directing transcriptional activity (Ciaccio et al., 1996). This 5′-flanking region contains a CTT-repeat element, two GC-boxes, and a reverse CCAAT-box. The GC- and CCAAT-boxes generally work as promoter signals in many eukaryotic cells. There is one study showing the Sp family of transcription factors play an important role in regulating constitutive expression of the AKR1C3 (Qin and Rosenfield, 2005).

The overall aim of this study is to increase the understanding on how AKR1C3 is genetically regulated and its putative role in prostate cancer. More specifically the aims of this study are to investigate (1) the influence of the CCAAT and the GC-elements

on the basal expression of the human AKR1C3 gene in HepG2 cells, (2) investigate if DHT affect the transcriptional activity of AKR1C3 in relation to the promoter A>G polymorphism, and (3) the allele frequency of this promoter SNP in relation to risk for prostate cancer in a Swedish population.

MATERIALS AND METHODS

SITE-DIRECTED MUTAGENESIS

Mutations in the CCAAT and GC-boxes were introduced using the pGLAKR1C3prom construct (Jakobsson et al., 2007) as template and the antisense primers (5′-gatggttaacatctggcatgtag-′3) and (5′-gaaacccctcccaacacccctg-′3) including the mutations together with a sense primer (5′-ggttgctatttgttctacaaa-′3). The PCRs were performed in 2 mM $MgCl_2$, 0.15 mM dNTP, 0.3 μl Taq Polymerase, 20 pmol of each primer, 100 ng of template, and were carried out in 30 cycles, each involving denaturing at 94°, 45 s, annealing at 53°, 45 s, elongation at 72°, 1 min, followed by 7 min elongation. The PCR products were gel-purified (Qiagen) and used as a mega primer together with an antisense primer (5′-cattccctgtcacttgtctg-′3) in a second PCR. The product was subcloned into a TA-vector (Invitrogen) and cleaved with the restriction enzymes, *Xho*I and *Kpn*1 (New England BioLabs), gel-purified, and subcloned into similar digested pGL3 Basic vectors (Promega). All the mutations were verified by sequence analysis.

CELL CULTURE

Human hepatoma HepG2 cells were maintained at 37°C in 5% CO_2 in MEM, 10% bovine serum, 2 mM glutamine, 1 mM sodium pyruvate, non-essential amino acids, 100 U penicillin, and 100 μg streptomycin/ml.

Drosophila DSL2 cells were maintained at room temperature in Schneider's Medium, 10% bovine serum, 2 mM glutamine, 100 U penicillin, and 100 μg streptomycin/ml.

All cell culture media and their ingredients were obtained from GIBCO/BRL (Gaithersburg, MD, USA).

TRANSFECTION ASSAY

HepG2 cells were plated in 35 mm 6-well plates at 2×10^5/well and incubated overnight. The cells were transfected using 15 μl Lipofectin (Life Technology), 2.5 μg pGLAKR1C3 Basic plasmid, and 2.5 μg β-gal control vector (Promega). Cells were incubated for 5 h at 37°C, the transfection solution was then replaced with fresh media. DSL2 cells were plated in 36 mm 6-well plates at 2×10^6 cells/well at the day of transfection. Two micrograms of pGLBasicAKR1C3 plasmid, 2.5 μg β-gal control vector (Promega), 0.5–1.5 μg of Sp1, and/or Sp3 expression vectors (pPACSp1 and/or pPACSp3 kindly provided by Dr. Ahmed Zaid) and 10 μl Superfect (Qiagen) were used in each transfection reaction. Cells were incubated for 3 h at room temperature, the transfection solution were then replaced with fresh media. Cells were harvested 48 h after transfection and luciferase activity was determined using luciferase assay reagent (Promega). βgal activity was measured using *ortho*-nitrophenyl β-D-galactopyranoside (Sigma) as a substrate. All values were corrected for endogenous galactosidase activity. The luciferase values were divided by the βgal value in order to correct for transfection efficiency.

REAL-TIME PCR

Total RNA was extracted from DHT exposed HepG2 cells with Trizol reagent (Invitrogen) according to the manufacturers manual. 1 μg RNA was reverse transcribed using First-strand cDNA Synthesis Kit (Amersham) with a random hexamer oligonucleotide. Real-time PCR was performed using 1 μl cDNA, 10 pmol AKR1C3 specific primers (Bogason et al., 2011), SYBR Green (Applied Biosystems), and quantified in an ABI Prism 700. Dilutions of a plasmid containing AKR1C3 cDNA (19T7-AKR1C3Glu77; Jakobsson et al., 2007) was used to construct a standard curve. For normalization of cDNA input in each PCR, 18S mRNA was used as an endogenous control.

HUMAN STUDY

In another part of the study a population based case–control study of 337 Caucasians living in the county of Örebro in Sweden were investigated. The cases were 176 patients, age 51–79 years, with prostate cancer, who were recruited consecutively between May 1994 and February 1996. In all, 81% agreed to participate. The controls were men, who were randomly selected every 3 months from the county population register and frequency matched for age. They were asked to participate by mail and 161 individuals agreed to participate, giving a response rate of 79%. Further description of the study population has been already published since this study population has been utilized in the evaluation of other polymorphisms involved in the androgen metabolism (Wadelius et al., 1999; Söderström et al., 2002). The Ethics Committee of the Regional Hospital of Örebro/Uppsala University, Sweden approved the study and all patients gave informed consent to participate. Genotyping of the A>G promoter polymorphism was performed as described previously (Jakobsson et al., 2007).

DATA ANALYSIS

Data of promoter activity and real-time PCR are expressed as mean ± SD Significance was assessed using two-tail Student's *t*-test using GraphPad Prism software v4.03 (San Diego, CA, USA). Genotype associations were assessed with binary logistic regression using Minitab statistical software package (v 12.1, Minitab Inc., State College, PA, USA). ORs were used as an approximation of relative risk, using 95% confidence intervals.

RESULTS

FUNCTIONAL ANALYSIS OF HUMAN AKR1C3 BASAL PROMOTER ACTIVITY

Transfection assay with wildtype and mutant reporter constructs were performed in HepG2 cells to study the effect of the CCAAT- and GC-box mutations on luciferase activity. Mutation of the CCAAT-box did not inhibit the promoter activity in HepG2, instead a borderline ($p = 0.06$) significant 50% increase was observed (**Figure 1**).

Transfections with the reporter construct including a mutated GC-box indicate that the promoter activity decreased significant (70%, $p < 0.05$) in HepG2 cells (**Figure 1**). These results indicate that the GC-box is important for directing and activating the transcription of the AKR1C3 gene in HepG2 cells.

Transfections with the wildtype promoter construct in DSL2 cells show that when increasing concentrations of Sp3 (0.5, 1.0, and

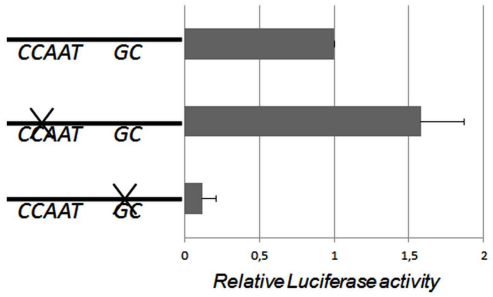

FIGURE 1 | Human AKR1C3 promoter fragment was subcloned into the pGL3 Basic vector and the CCAAT- and GC-elements were mutated and transiently transfected into HepG2 cells. The bar graph represents mean ± SD from at least three experiments. The luciferase expression was normalized to the transfection efficiency by β-galactosidase expression. When the CCAAT-box was mutated there was a borderline significant 50% increase in the relative luciferase activity. When the GC-box was mutated the luciferase activity significantly decreased indicating that the GC-box is required for the basal transcription of the AKR1C3 gene in HepG2 cells.

1.5 μg) were added to the cells, the promoter activity increased 1.5 times, whereas no activation of the basal activity of shown when increasing concentrations (0.5, 1.0, and 1.5 μg) of Sp1 were added (**Figure 2A**).

When Sp3 was added at 1 μg the addition of Sp1 (1 μg) did not further activate the promoter activity, whereas a Sp3 induced the promoter activity 2.2 ($p < 0.05$) times when added together with Sp1 (**Figure 2B**).

These results indicate that both the Sp1 and Sp3 proteins are involved in the transcription of AKR1C3 gene, although the Sp3 seem to be the most important protein.

STEROID AND PROMOTER ACTIVITY IN RELATION TO PROMOTER POLYMORPHISM

When the HepG2 cells were exposed to high concentration (25 μM) of DHT over night the promoter activity of the wildtype (A) construct increased 2.2-fold ($p = 0.048$), whereas for the polymorphic construct (G) there were no significant induction observed (**Figure 3A**). When HepG2 cells were exposed to DHT a significant sevenfold increase of the AKR1C3 mRNA levels ($p = 0.038$) was found (**Figure 3B**).

PROMOTER POLYMORPHISM AND PROSTATE CANCER RISK

The distribution of the promoter polymorphism was in Hardy–Weinberg equilibrium. The allele frequency of the promoter polymorphism G in healthy participants were 39% (**Table 1**), as in agreement with previous studies in Caucasians (Jakobsson et al., 2007). Individuals displaying an A-allele (A/G and A/A) showed 0.59 times decreased risk for prostate cancer compared to individuals homozygous for the G-allele (**Table 2**). The results were quit not significant ($p = 0.058$).

DISCUSSION

To evaluate the possible role for AKR1C3 in prostate cancer, a promoter polymorphism (A>G, rs3763676) were genotyped in a population based case–control study. The result indicates that the promoter SNP in the AKR1C3 gene may modulate the prostate cancer risk and that there may be an advantage to have an A-allele. The results are in agreement with our *in vitro* finding that the promoter activity of the wildtype A variant is induced by DHT and with our previous finding that the G-variant displayed lower transcription activity in a prostate cancer cell line (Jakobsson et al., 2007). AKR1C3 is an important enzyme in the metabolism of DHT, particularly in the inactivation of DHT to the less potent androgen 3α-Adiol (Lin et al., 1997). It is possible that A-carriers may have an improved protection against high concentrations of DHT since this allele is correlated with higher transcriptional activity, and is induced by DHT. Contradictory to this hypothesis, i.e., that high expression of AKR1C3 is protective against androgen load, is a study by Stanbrough et al. (2006) who found a 5.2-fold increase of AKR1C3 mRNA level in androgen-independent prostate cancer bone metastasis.

Recent studies have shown that another AKR1C3 polymorphisms (c90 G/A (rs7741), known to be in linkage disequilibrium with the A>G promoter polymorphism ($R^2 = 1$; International HapMap Consortium, 2005), is associated with prostate disease. The c90 A-allele has been associated with increased risk for prostate enlargement (Roberts et al., 2006), and increased risk of both familial and sporadic prostate cancer. (Cunningham et al., 2007). Additionally, another AKR1C3 SNP (rs4881400), not in linkage disequilibrium with the promoter polymorphism, has been associated with prostate cancer risk (Kwon et al., 2012). Thus it is likely that AKR1C3 play a role in the etiology of prostate related diseases. Whether the promoter polymorphism investigated in this study is associated with prostate cancer risk needs to be further investigated in larger prostate cancer case–control studies. The fact that the promoter SNP studied herein is included in common GWAS analysis such as Affymetrix SNP 6.0, will increase the chance to find such association.

In agreement with our results, one study found that the G-allele was significantly more frequent in women with PCOS. Like prostate cancer, PCOS is also considered to be an androgen dependent disease, and the authors speculated that the increased risk may be due to higher plasma testosterone levels in subjects homozygous for G-allele (Qin et al., 2006). However, a subsequent study were not able to find an association between the promoter A>G polymorphism and PCOS (Goodarzi et al., 2008).

Here we show that DHT increase the promoter activity of the AKR1C3 promoter including the wildtype (A) sequence, whereas the polymorphic G-variant was not up-regulated in HepG2 cells. Moreover, the AKR1C3 mRNA level was induced sevenfold after DHT exposure in HepG2 cells. This finding was not in agreement with a study performed in LNCaP cells, where DHT was shown to decrease the expression of AKR1C3 by 70% (Wang and Tuohimaa, 2007). This discrepancy of may be due to the different DHT concentrations used (10 nM versus 25 μM) in the studies. The former concentration is reflecting the physiological of DHT in the circulation, whereas the supra-physiologic concentration of 25 μM reflects the high levels of DHT produced locally in the prostate (Olsson et al., 2011). Moreover, the androgen response may differ between the cell-lines used. Further investigations are

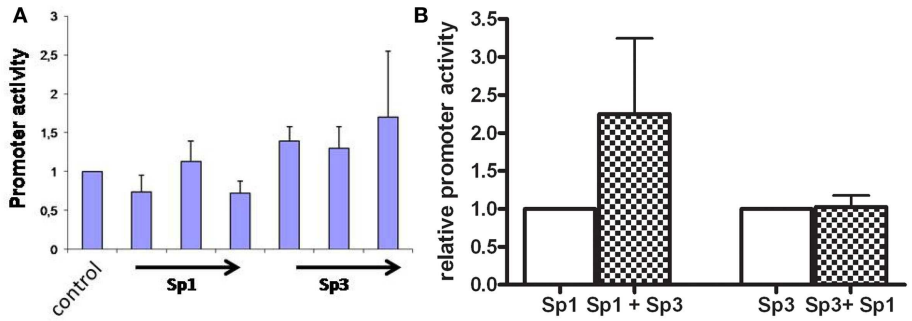

FIGURE 2 | (A) Transcriptional activities of the AKR1C3 promoter construct transiently transfected into DSL2 cells. The DSL2 cells were cotransfected with Sp-proteins in increasing concentrations. The bar graph represents mean ± SD from at least three experiments. The luciferase expression was normalized to the transfection efficiency by β-galactosidase expression. Control background activity was increased twofold when Sp3 (0.5, 1, and 1.5 μg) were added to cells, whereas no significant affect was observed when increasing concentrations of Sp1 were added. **(B)** Human AKR1C3 promoter construct was transiently transfected into HepG2 cells cotransfected with Sp1/Sp3 proteins. The bar graph represents mean ± SD from at least three experiments. The luciferase expression was normalized to the transfection efficiency by β-galactosidase expression. When the Sp1 protein (1 μg) containing DSL cells were cotransfected with Sp3 (1.5 μg) the promoter activity increased 2.2-fold. When the Sp3 (1 μg) containing DSL cells were cotransfected with Sp1 (1.5 μg) no induction in promoter activity was observed.

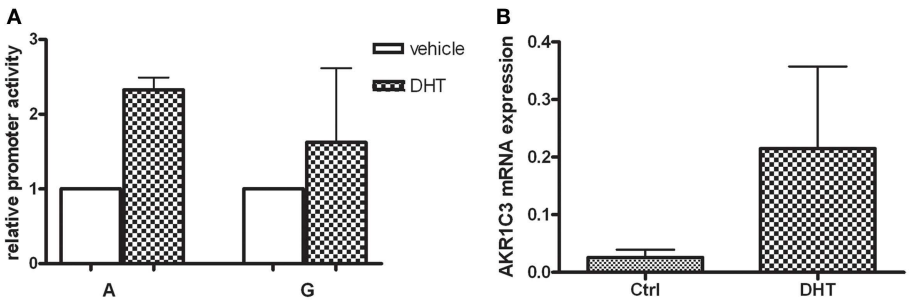

FIGURE 3 | (A) The HepG2 cells were transfected with the wildtype AKR1C3 promoter (A) and the polymorphic (G) construct and exposed to 25 μM DHT over night. The bar graph represents mean ± SD from at least three experiments. The luciferase expression was normalized to the transfection efficiency by β-galactosidase expression. After DHT treatment and promoter activity of the wildtype (A) significantly increased whereas no significant alteration was observed for the polymorphic (G) construct. **(B)** The mRNA expression of the human AKR1C3 gene in HepG2 cells was evaluated using real-time PCR. Significant increase in AKR1C3 mRNA levels was observed after incubation with 25 μM DHT over night. The bar graph represents mean ± SD from four experiments.

Table 1 | Genotype and allele frequencies of the promoter polymorphism (A>G) in a Swedish population sample of prostate cancer and controls.

	Cases % (*n*)	Controls % (*n*)
AA genotype	40 (71)	39 (63)
GG genotype	19 (33)	12 (19)
AG genotype	41 (72)	48 (77)

Table 2 | Prostate cancer risk estimates for AKR1C3 A/G promoter polymorphism among Swedish Caucasian men.

Genotype	Odds ratio	95% CI
G/G	1.00	Ref.
A/G	0.54	(0.28–1.03)
A/A	0.65	(0.34–1.25)
A*	0.59	(0.32–1.08)

**All carriers of A compared to homozygous carriers of G.*

required to evaluate how androgens modulate expression and activity of AKR1C3 in different cell types, particularly in androgen sensitive cells.

In order to study the cis-acting elements involved in the AKR1C3 basal promoter activity we transfected the promoter wildtype and two mutated (CCAAT- and GC-elements) promoter construct in HepG2 cells. The result indicates that the inverted CCAAT-box is not involved in the basal transcription of AKR1C3. Instead binding to the CCAAT-box may have an inhibitory effect on the AKR1C3 expression since we observed an increase in promoter activity when this element was mutated. The GC-box on the other hand appears to be involved in the constitutive expression. The GC-boxes are GC rich sequences recognized by the Sp transcription factor family (Kingsley and Winoto, 1992). To

further examine the trans-acting proteins involved in the AKR1C3 transcription, the wildtype promoter construct was transfected into DSL2 cells. DSL2 cells are used to specifically study the Sp family of transcription factors since these cells lack Sp-proteins. When constructs were added to the cells without the Sp1/Sp3 expression vectors, a low background activity was observed. The addition of Sp3 increased the expression, whereas the addition of Sp1 alone did not induce the promoter activity. Co-transfection with Sp1 and Sp3 proteins also indicate that Sp3 may have a more impact on the promoter activity compared to Sp1.

Previous studies of the AKR1C3 promoter have shown that a 169-bp region (−104 to +65) is capable of directing transcriptional activity (Ciaccio et al., 1996). This 5′-flanking region contains a CCT repeat element, 2 GC-boxes and a reverse CCAAT-box. Qin and Rosenfield (2005) showed that the binding of Sp1/Sp3 to the CCT repeat element was important for the constitutive and forskolin stimulated AKR1C3 promoter activity in H295R cells. The GC- and CCAAT-boxes generally work as promoter signals in many eukaryotic cells. The GC-boxes are GC rich sequences recognized by the Sp transcription factor family (Kingsley and Winoto, 1992). Our results together with Qins (Qin and Rosenfield, 2005) clearly show that the transcription of AKR1C3 gene is driven by Sp-proteins.

In conclusion, our results further support previous findings that AKR1C3 play an important role in the androgen metabolism and in the etiology of prostate cancer.

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