



# RARG Gene Dysregulation in Acute Myeloid Leukemia

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Retinoic acid receptor  $\gamma$  (RAR $\gamma$ ) belongs to the nuclear receptor superfamily and shares 90% homology with retinoic acid receptor  $\alpha$  (RAR $\alpha$ ) and retinoic acid receptor  $\beta$  (RAR $\beta$ ). *RARA* rearrangements are well-known to be involved in acute promyelocytic leukemia (APL), but *RARG* rearrangements can also resemble this kind of leukemia. In this review we trace the role of RAR $\gamma$ , considering both its physiological and oncogenic contribution; from 2011 to date, nine cases of patients harboring *RARG* fusions have been reported. These patients showed typical APL features, including the clinical presentation, coagulation abnormalities and morphological features of bone marrow (BM), but are not responsive to APL standard therapy. We stress the urgent need for a better comprehension of the critical role of *RARG* dysregulation in the leukemogenesis process, since optimum therapy strategies have not yet been established.

**Keywords:** retinoic acid receptor  $\gamma$ , acute promyelocytic leukemia, acute myeloid leukemia, gene fusions, protein fusions

## INTRODUCTION

Acute myeloid leukemia (AML) is a hematologic malignancy that may arise in patients with another hematological disorder already present or after previous therapy, for instance following previous exposure to alkylating agents or DNA topoisomerase inhibitors or radiation (Sill et al., 2011), but most frequently it appears as a de novo malignancy in previously healthy individuals (De Kouchkovsky and Abdul-Hay, 2016). The bases of the pathogenesis reside in an abnormal and malignant clonal expansion of specific progenitor myeloid cells that do not complete their physiological differentiation process (De Kouchkovsky and Abdul-Hay, 2016). The cause is to be found in a genetic alteration of the progenitor hematopoietic cell that gives life to a clonal population of blast cells, with the manifestation of an atypical hematopoiesis (Levine, 2013; Thomas and Majeti, 2017). Already in 1957, APL accounted for 5–15% of the total AML cases (Sztokowski et al., 2015). In fact, APL is a different subtype of AML characterized by an accumulation and expansion of leukemic cells that fail to go beyond the promyelocyte stage of myelopoiesis (de Thé et al., 1991). Before the discovery of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), patients with APL had a high risk of mortality, due to hemorrhagic complications before and during induction therapy (Choudhry and DeLoughery, 2012). Both ATRA and ATO behave as differentiating agents, since they promote the differentiation and maturation of leukemic promyelocytes to neutrophils (Asou et al., 1998; Karim et al., 2014; Daver et al., 2015). According to the current WHO criteria, the presence of *t*(15;17)(q22;q12), hence of the promyelocytic leukemia protein (*PML*)-*RARA* gene fusion, allows an AML to be classified as APL, but other variant *RARA* translocations with other partner genes are not only considered distinct, but not all have typical APL features and indeed, some patients show resistance to ATRA (Arber et al., 2016). In particular,

in a subset of patients the *PML-RARA* fusion is not detected, but other rearrangements are identified that involve *RARB* and *RARG*, two distinct isoforms of *RARA*, that cause a pathological phenotype that resembles APL (Marinelli et al., 2007; Osumi et al., 2018). The aim of this review is to focus on the various emerging cases of APL without the *RARA* gene rearrangement and with the *RARG* gene rearrangement or dysregulation that presented abnormal promyelocytes fully in accordance with the APL phenotype.

## THE MOLECULAR BIOLOGY OF RETINOIC ACID RECEPTOR $\gamma$ (RAR $\gamma$ )

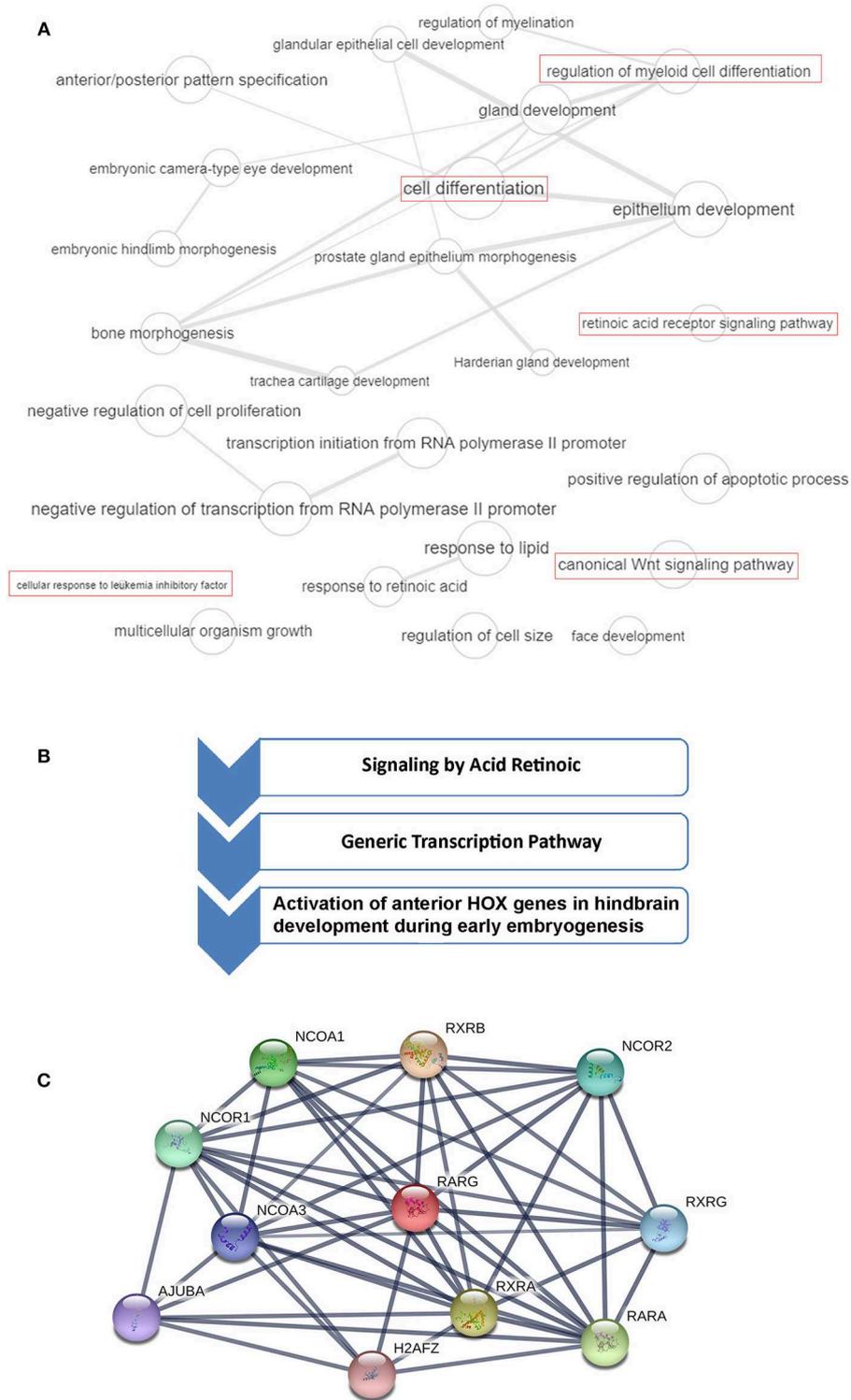
RAR $\gamma$  belongs to the nuclear receptor superfamily and shares 90% homology with RAR $\alpha$  and RAR $\beta$  (Chambon, 1996). These three types of RARs have been discovered by cDNA cloning in both human and mouse and were identified as the only three members of the RAR family in mouse and man (Kastner et al., 1994). Moreover, all three RAR types have been characterized in the newt (Giguère et al., 1989; Ragsdale et al., 1989), RAR $\beta$  cDNA has been cloned from chicken (Noji et al., 1991; Padanilam et al., 1991; Rowe et al., 1991; Smith and Eichele, 1991), RAR $\alpha$  and RAR $\gamma$  cDNA have been found in *Xenopus* (Ellinger-Ziegelbauer and Dreyer, 1991; Blumberg et al., 1992) and have been isolated from zebrafish (Kastner et al., 1994), so these findings suggest that this RAR genes family exists in all vertebrates, mediating a series of effects, that may sometimes even be conflicting. RAR $\gamma$  is involved in several biological processes and cellular pathways, interacting with different proteins (Figure 1). The chromosomal mapping of *RARG* in humans is on 12q13, while *RARA* and *RARB* mapping is on 17q21.1 and 3p24, respectively (Mattei et al., 1988, 1991; Ishikawa et al., 1990). The protein structure of RAR $\gamma$  presents a DNA binding domain (DBD) that receives two “zinc fingers” that allow the protein to bind to specific DNA sequences in the promoter of their target genes, known as retinoic acid response elements (RAREs), and a ligand-binding domain (LBD) that confers the ligand binding specificity, assuming a ligand-inducible transactivation function (Kastner et al., 1990; Leroy et al., 1991; Zelent et al., 1991; Liu and Linney, 1993; Bastien and Rochette-Egly, 2004; Storlazzi et al., 2007; Al Tanoury et al., 2013). In detail, it is possible to recognize six different regions in the RAR primary sequence (A-F), which exhibit differential degrees of conservation (Kastner et al., 1994). The most conserved regions among the three RARs, within a given species, are the DBD (region C), the

LBD (region E) and the region B which presents a promoter-specific transcription activation function (Nagpal et al., 1992). Although the D region, known as a hinge region, is well-conserved in its N-terminal portion, the central region appears to be more variable. Finally, the A and F regions differ notably among the three receptors (Kastner et al., 1994). For each RAR isotype it is possible to identify two or more isoforms; for regarding RAR $\gamma$ , two main isoforms (RAR $\gamma$ 1 and RAR $\gamma$ 2) have been identified, that show a different spatio-temporal expression. RAR $\gamma$ 1 is found in the later phase of embryogenesis, as well as in the skin of newborns and adults, while RAR $\gamma$ 2 is expressed in the early embryo (Kastner et al., 1990; Chambon, 1993). In the presence of a ligand, such as ATRA, RARs form heterodimers with the retinoid X receptors (RXRs) that operate as transcription factors, activating the RAREs regions in the target genes promoter (Mic et al., 2003; Bastien and Rochette-Egly, 2004). In detail, each RARs subtype presents a different sensitivity at different concentrations of ATRA: RAR $\alpha$  activation occurs in the presence of high concentrations of ATRA, whereas RAR $\gamma$  activation requires the lowest amount of ligand (Beard et al., 2002).

## INVESTIGATION OF RAR $\gamma$ ROLE

Although knockout mice studies have shown some functional redundancy among the different RAR isotypes, in reality each of them has a unique role in the processes of development and differentiation that cannot be replaced by the action of the other isotypes (Taneja et al., 1995; Mark et al., 2006). *RARG* null mice display growth deficiency (Lohnes et al., 1993); indeed, RAR $\gamma$  is highly expressed in the growth plate and its absence is associated with a reduced chondrocyte proliferation and decreased expression and deposition of proteoglycans (Williams et al., 2009). In addition, RAR $\gamma$  is required for the correct formation of the axial skeleton including anteriorization of the cervical and thoracic vertebrae (Lohnes et al., 1993; Wendling et al., 2001). Other studies have demonstrated that the genetic ablation of *RARG* in mice induced a decrease in bone mass due to an increase in osteoclastogenesis and consequent loss of trabecular bone mass (Walkley et al., 2007; Green et al., 2015). In addition, RAR $\gamma$  is crucial for the formation of normal alveoli and alveoli elastic fibers in the lung (McGowan et al., 2000) and its genetic ablation is associated with male sterility and with alterations of the prostatic glandular epithelia (Lohnes et al., 1993). Moreover, RAR $\gamma$  signaling is critical for epigenetic changes induced by retinoic acid (RA) (Kashyap et al., 2013). RAR $\gamma$ , as well as RAR $\alpha$ , are crucial for hematopoietic development (Purton, 2007). Indeed, while RAR $\alpha$  induces granulocytic differentiation, RAR $\gamma$  plays a central role in maintaining the balance between the self-renewal state of hematopoietic stem cells (HSCs) and their differentiation (Purton et al., 2006; Purton, 2007). Purton et al. reported that *RARG* null mice show reduced numbers of long-term repopulating HSCs and increased numbers of more committed hematopoietic progenitors, therefore *RARG*<sup>-/-</sup> BM displayed significantly increased numbers of common myeloid progenitors

**Abbreviations:** RAR $\gamma$ , retinoic acid receptor  $\gamma$ ; RAR $\alpha$ , retinoic acid receptor  $\alpha$ ; RAR $\beta$ , retinoic acid receptor  $\beta$ ; APL, acute promyelocytic leukemia; BM, bone marrow; AML, acute myeloid leukemia; ATRA, all-trans retinoic acid; ATO, arsenic trioxide; PML, promyelocytic leukemia protein; DBD, DNA binding domain; RAREs, retinoic acid response elements; RXRs, retinoid X receptors; RA, retinoic acid; HSCs, hematopoietic stem cells; PB, peripheral blood; NUP98, nucleoporin 98; CR, complete remission; WT1, Wilms' tumor; Allo-HSCT, allogeneic HSC transplantation; CPSF6, Cleavage and polyadenylation specific factor 6; OS, overall survival; LFS, leukemia free survival; EIF4B, eukaryotic translation initiation factor 4B; NPM1, nucleophosmin 1; bcr3, breakpoint cluster region 3; EZH2, enhancer of zeste 2 polycomb repressive complex 2 subunit; DNMTs, DNA methyltransferases.



**FIGURE 1 |** *RARG* gene ontology (GO), cellular pathways and protein-protein interaction analysis. **(A)** GO terms of *RARG* visualized by REVIGO (<http://revigo.irb.hr/>). Bubble size indicates the frequency of the GO term in the Gene Ontology Annotation database. Lines in the graph link highly similar GO terms, where their width indicates the degree of similarity. In red are highlighted *RARG* cellular processes that may be altered in leukemia. **(B)** *RARG* main cellular pathways derived from reactome database (<https://reactome.org/>). **(C)** *RARG* protein-protein interactions obtained by STRING database (<https://string-db.org/>). The interactions include direct (physical) and indirect (functional) associations.

and common lymphoid progenitors, unlike *RARG*<sup>+/+</sup> BM. This finding suggests that loss of *RARG* induces an imbalance in HSC self-renewal decisions, supporting differentiation divisions. Moreover, Purton et al. have shown that RAR $\gamma$  signaling affected other genes known to elicit HSC self-renewal, such as *NOTCH1*, whose expression decreases severely in the absence of RAR $\gamma$  (Purton et al., 2006). Walkley et al., showed that *RARG* null mice exhibit a considerable increase in granulocytes in the peripheral blood (PB), in the BM and spleen, developing a myeloproliferative-like syndrome and displaying defects in both erythropoiesis and in B lymphopoiesis (Walkley et al., 2007; Dewamitta et al., 2014). Transplantation studies have confirmed that the expression of RAR $\gamma$  is essential in the BM microenvironment for a correct hematopoiesis to occur (Joseph et al., 2016). Indeed, Dewamitta et al. suggested that deficiencies observed in *RARG*<sup>-/-</sup> mice are due to an aberrant *RARG*<sup>-/-</sup> BM microenvironment, and therefore it is not an intrinsic cellular defect (Dewamitta et al., 2014). Green et al. also confirmed that RAR $\gamma$  is a crucial regulatory key for the presence of a healthy BM microenvironment, since mice with conditionally deleted *RARG* in more primitive limb bud-derived mesenchymal stem cells and their progeny, achieved through the use of *Prrx1-Cre*, showed alterations in the number of both osteoclasts and osteoblasts, with consequent modifications in the trabecular bone and an abnormal angiogenesis and B lymphopoiesis. These results seem to confirm the key role of RAR $\gamma$  in maintaining homeostasis in the various processes that occur in the BM microenvironment (Conserva et al., 2019), such as endochondral bone formation, angiogenesis, osteoclastogenesis and B lymphopoiesis (Green et al., 2018). Considering that vitamin A-derived retinoids play a central role in the growth and differentiation of a variety of cell types, RAR $\gamma$  in particular mediates various anti-proliferative and apoptotic effects of retinoids in certain tissues and cancer cells, such as melanoma and neuroblastoma cells (Spanjaard et al., 1997; Meister et al., 1998). Chen et al. also showed that the genetic ablation of *RARG* in a model of epidermal tumorigenesis enhanced the tumor incidence of Ras-transformed keratinocytes and was associated with retinoids resistance (Chen et al., 2004). Several studies, in recent years, have described various cases of patients carrying translocations involving *RARG* and showing a leukemic phenotype that resembles APL (Table 1). Therefore, it seems that *RARG*, just like *RARA*, can somehow rearrange and mediate oncogenic effects.

## RARG IN MYELOID MALIGNANCIES

*RARG* rearranged leukemia is a rare specific subtype of AML, but its characterization is very ambiguous because its features are so strongly similar to APL. Indeed, patients presenting *RARG* rearrangements share typical APL clinical presentations, coagulation abnormalities and morphological and immunophenotypic features of BM (Luo et al., 2019). *RARA* rearrangements are known to generate APL, and rearrangements involving *RARB* or *RARG* can resemble APL. Typical APL is

characterized by recurrent *PML-RARA* expression, but up to now, to our knowledge, eleven cases with *RARG* dysregulation have been identified (Such et al., 2011; Ha et al., 2017; Liu et al., 2018; Miller et al., 2018; Qin et al., 2018; Chen et al., 2019; Luo et al., 2019; Zhang et al., 2019b) (Figure 2). As previously reported by Conserva et al., the three RARs are highly homologous so it is not unexpected that rearrangements involving both *RARB* and *RARG* can generate similar diseases (Conserva et al., 2019). However, the involvement of *RARG* principal cellular pathways in AML needs to be further clarified (Figure 1A).

## NUP98-RARG

The first AML patient with a *RARG* rearrangement, *NUP98-RARG*, was reported in 2011 (Such et al., 2011). *NUP98* is the nucleoporin 98 gene mapped on chromosome 11p15, frequently involved in several rearrangements in both myeloid and lymphoid malignancies (Romana et al., 2006; La Starza et al., 2009). The patient (no. 1, Table 1) was a 35-years-old man exhibiting morphologic and clinical characteristics resembling APL. The genomic aberration found was translocation *t*(11;12)(p15;q13), plus a 1.0 Mb microdeletion in 11p15 and a 2.5 Mb microdeletion in 12q13. *NUP98* exon 12 was fused in frame to *RARG* exon 4. Interestingly, the *NUP98* 5' region encodes Gly-Leu-Phe-Gly-repeats (GLFG) and GLE2p-binding sequence (GLEBS)-like motifs, which act as transcription activators, providing docking sites for the nuclear transport of RNA and protein between the nucleus and the cytoplasm; while the 3' region *RARG* involved in the fusion includes DBD and LBD. The mechanism underlying the transformation mediated by *NUP98-RARG* is unknown. The patient was started on ATRA treatment that was soon discontinued and switched to a standard 7 + 3 schedule (cytarabine and idarubicin) as induction therapy, then he achieved complete remission (CR). Two years later, the patient relapsed; he achieved second CR but then he died due to an infection complication (Such et al., 2011). Several studies were conducted by Qiu et al. focused on the RAR $\gamma$  involvement in retinoid signaling. Using green fluorescent protein-tagged versions of these proteins and analyzing their signal, they demonstrated that the novel *NUP98-RAR $\gamma$*  fusion protein acquires a different subcellular localization compared to *NUP98* and wild-type RAR $\gamma$ . This localization is strongly dependent on the RAR $\gamma$  DBD domain, as shown by different experiments conducted using mutant variants of *NUP98-RAR $\gamma$* . They also verified, through co-immunoprecipitation assays, the *NUP98-RAR $\gamma$*  ability to form aberrant homo-dimers and homo-oligomers, but this feature is granted by the *NUP98* portion of the chimeric protein. The RAR $\gamma$  fraction is essential for interacting with RXR $\alpha$ : they also demonstrated that the RXR agonist can suppress the *NUP98-RAR $\gamma$ /RXR* connection. Luciferase reporter experimental results also suggested that *NUP98-RAR $\gamma$*  may have equivalent transcriptional properties to those of the RAR $\alpha$  fusion protein, involved in APL, on its downstream targets. The oncogenic potential of *NUP98-RAR $\gamma$*  was confirmed by retroviral transduction/transformation assay in murine HSCs; furthermore, the cellular transformation

**TABLE 1 |** AML with *RARG* rearrangements updated reported cases.

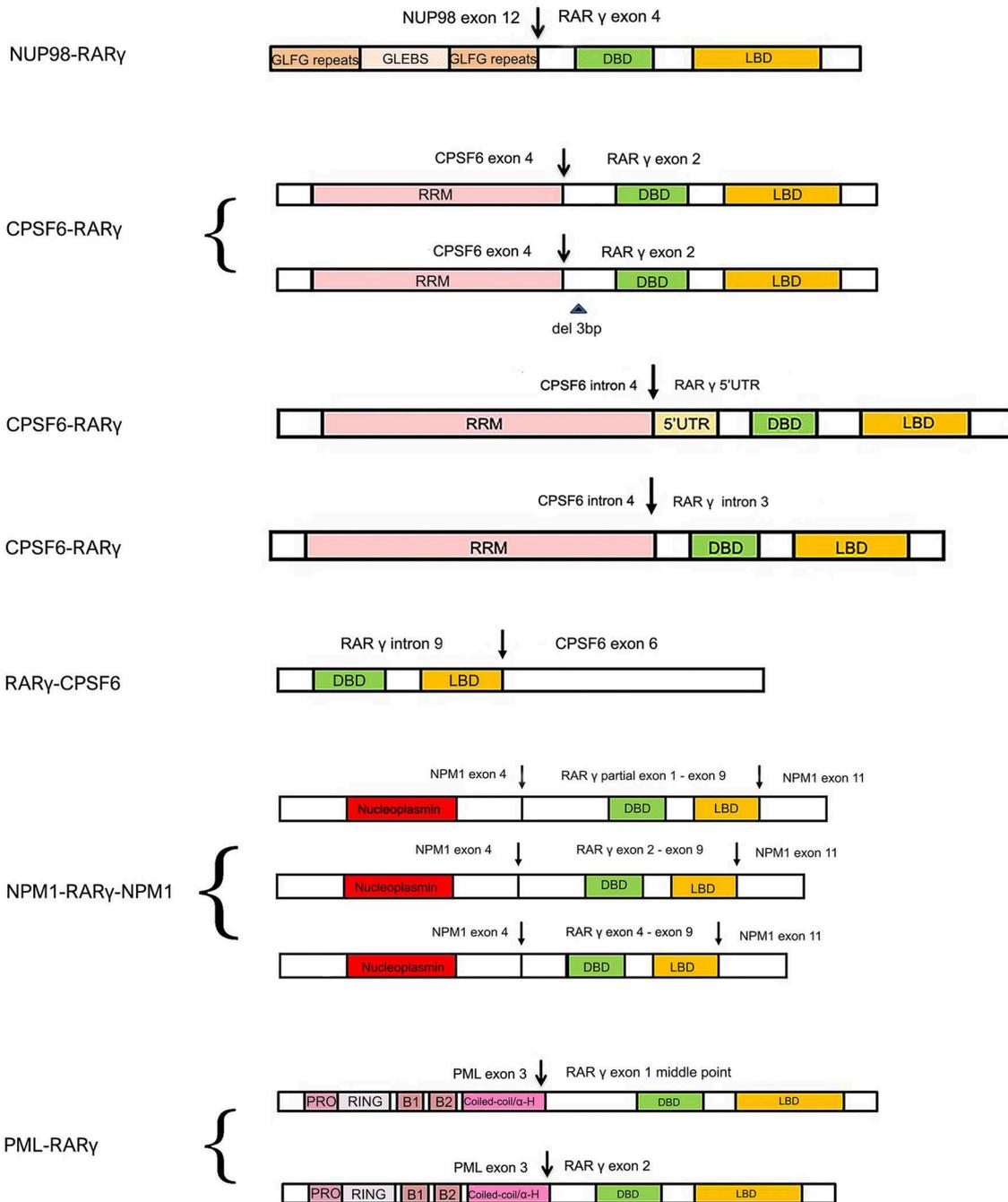
No.	Age/ gender	PB	Morphology	Cytogenetics	Molecular analysis	Therapy	ATRA responsivity	References
1	35/M	WBC: 12 × 10 <sup>9</sup> /L HB: 6.0 g/dL PLT: 8 × 10 <sup>9</sup> /L	80% hypergranular promyelocytes with Auer rods	46, XY t(11;12)(p15;q13)[16] /46, XY[4]	<i>NUP98-RARG</i>	IA as induction therapy, chemotherapy followed by auto-HSCT as consolidation therapy	None	Such et al., 2011
2	45/F	WBC: 0.2 × 10 <sup>9</sup> /L HB: 66g/L PLT: 60 × 10 <sup>9</sup> /L	94.5% hypergranular promyelocytes with Auer rods	46, XX t(11;12)(p15;q13)[16]	<i>NUP98-RARG</i> <i>WT1</i> mutation	ATRA + ATO treatment, switched to IA as induction therapy	None	Luo et al., 2019
3	22/M	WBC: 112.6 × 10 <sup>9</sup> /L HB: 82 g/L PLT: 92 × 10 <sup>9</sup> /L	91.0% promyelocytes	46, XY t(11;12)(p15;q13)	<i>NUP98-RARG</i> <i>WT1</i> mutations	ATRA, ATO, +idarubicin, then HAA, DA as induction therapy	None	Zhang et al., 2019b
4	38/M	WBC: 1.68 × 10 <sup>9</sup> /L HB: 8.0 g/dL PLT: 79 × 10 <sup>9</sup> /L	65% promyelocytes	46, XY[20]	<i>CPSF6-RARG</i> <i>WT1</i> mutation	ATRA + RIF d1-25, MA	None	Qin et al., 2018
5	48/F	WBC: 0.81 × 10 <sup>9</sup> /L HB: 4.2 g/dL PLT: 92 × 10 <sup>9</sup> /L	89% hypergranular promyelocytes with Auer rods	92, XXXX[2]	<i>CPSF6-RARG</i> <i>DNMT3A</i> mutation	ATRA + ATO + IDA, IAG + ATRA + ATO, Decitabine as induction therapy	None	Liu et al., 2018
6	51/F	WBC: 20.15 × 10 <sup>9</sup> /L HB: 6.5 g/dL PLT: 45 × 10 <sup>9</sup> /L	87.5% hypergranular promyelocytes	46, XX, del(12)(p12)[2]/46, XX[18]	<i>CPSF6-RARG</i> <i>WT1</i> , <i>KRAS</i> mutation	ATRA + DNR, DA as induction therapy, HD-Ara-C followed by 3 + 7 regimens	None	Liu et al., 2018
7	26/M	WBC: 16.4 × 10 <sup>9</sup> /L HB: 10.5 g/dL PLT: 120 × 10 <sup>9</sup> /L	60% blasts and 15% promyelocytes	45, X, -Y [10]/45, idem, add(6)(q?13)[2]/46, XY[8]	<i>RARG-CPSF6</i> <i>BMPR1A</i> , <i>NEAT1</i> , <i>WT1</i> mutation	ATRA + IA as induction therapy	None	Miller et al., 2018
8	69/M	WBC: 1.5 × 10 <sup>9</sup> /L HB: 123 g/L PLT: 204 × 10 <sup>9</sup> /L	56% hypergranular promyelocytes	46, XY	<i>NPM1-RARG-NPM1</i>	ATO + ATRA treatment	None	Chen et al., 2019
9	64/F	WBC: 1.26 × 10 <sup>9</sup> /L HB: 8.4 g/dL PLT: 120 × 10 <sup>9</sup> /L	86.5% blasts and atypical hypergranular promyelocytes with Auer rods	46, XX, t(12;15)(q13;q22)	<i>PML-RARG</i>	IA as induction therapy, HD-Ara-C, followed by allo-HSCT as consolidation therapy	NA	Ha et al., 2017
10	42/M	WBC: 18,500/μL HB: 8.6 g/dL PLT: 118,000/μL	83% blast cells; among them 30% with hypergranulated cytoplasm with Auer rods and PCH anomaly	46, XY[20] der(2) chr. (17q21.32-q25.3 duplication and 2q35- 37.3 deletion)	<i>EZH2</i> mutation <i>RARA</i> and <i>RARG</i> downregulation	3 + 7 regimens, then FLAG-IDA	NA	Coccaro et al., 2018
11	33/F	WBC: 35.6 × 10 <sup>9</sup> /L HB: 76 g/L PLT: 67 × 10 <sup>9</sup> /L	4.0% myeloblasts and 70.5% aberrant promyelocytes	46, XX	<i>ELL-MLL</i> <i>RARG</i> upregulation <i>RARA</i> downregulation	ATO treatment	NA	Zhang et al., 2019a

No., number; PB, peripheral blood; ATRA, all-trans retinoic acid; Ref., reference; M, male; F, female; WBC, white blood cell; HB, hemoglobin; PLT, platelet; IA, Idarubicin and cytarabine; Auto-HSCT, autologous hematopoietic stem cell transplantation; ATO, arsenic trioxide; HAA, homoharringtonine, clacinomycin, and cytarabine; DA, Daunorubicin and cytarabine; RIF, Realgar-Indigo naturalis formula; MA, mitoxantrone and cytarabine; IDA, Idarubicin; IAG, Idarubicin, cytosine arabinoside, and G-CSF; DNR, Daunorubicin; HD-Ara-C, High-dose cytarabine; Allo-HSCT, allogeneic hematopoietic stem cell transplantation; FLAG-IDA, Fludarabine, Cytarabine, G-CSF, Idarubicin; NA, not available.

ability requires both RARγ and NUP98 contributions and these transformed cells are sensitive to RXR agonist and ATRA treatment (Qiu et al., 2015). It was necessary to conduct several experiments to test ATRA responsivity since the first patient with *RARG* rearrangement received ATRA simultaneously to chemotherapy, so it was difficult to evaluate the effective *in vivo* responsivity to ATRA, even if it has been reported that fusion proteins generated with RARγ confer responsiveness to ATRA *in vitro* and *in vivo* (Marinelli et al., 2007, 2009). The *in vitro* studies showed that the *NUP98/RARG* rearrangement confers ATRA resistance (Such et al., 2014). Moreover, another patient (no.

2, **Table 1**) with *NUP98/RARG* exhibited a primary resistance to ATRA treatment (Luo et al., 2019). Sequencing of the fusion transcript showed also that *NUP98* exon 12 was fused in frame to *RARG* exon 4, in the first patient described, and the Wilms' tumor (*WT1*) mutation was also identified. These studies point out an evident paradox of ATRA resistance/responsiveness *in vitro* and *in vivo*: it may depend on the culture assay condition or on the acquisition of additional mutations causing resistance (Such et al., 2014; Qiu et al., 2015). A third case of *NUP98-RARG* AML (no. 3, **Table 1**) was observed by Zhang et al. The break points were the same as in the other two cases reported in literature.

Fused Protein



**FIGURE 2 |** Schematic comparison of RAR $\gamma$  fusion proteins identified or expected in AML reported cases. The variably colored rectangles indicate all the important domains of RAR $\gamma$  and its partner proteins. The arrows indicate DNA break points. Curly brackets are used to group fusion proteins all found in one patient. GLFG, Gly-Leu-Phe-Gly; GLEBS, GLE2p-binding sequence; RRM, RNA recognition motif; PRO, proline-rich region; RING, RING finger; B1/B2, B-Box; Coiled-coil/ $\alpha$ -H, coiled-coil/ $\alpha$ -helical; NLS, nuclear localization signal.

In addition, two *WT1* mutations were found. Given the two previous cases, the author established that *NUP98-RARG* AML is sensitive to the standard 3+7 regimen, and that allogeneic

HSC transplantation (allo-HSCT) may be the best procedure to achieve CR. It is possible that additional genetic mutations can cause ATRA resistance (Zhang et al., 2019b).

## CPSF6-RARG and RARG-CPSF6

Cleavage and polyadenylation specific factor 6 (CPSF6) is a subunit of cleavage factor I, which is an RNA binding protein complex involved in the alternative cleavage and polyadenylation process (Millevoi and Vagner, 2010). Although several studies have demonstrated the involvement of CPSF6 in HIV infection and breast cancer (Rasheedi et al., 2016; Binothman et al., 2017), the role of CPSF6 in myeloid leukemia has not yet been clarified. The first case of *CPSF6-RARG* rearrangement was found by Qin et al. in an AML 38-years-old male (no. 4, **Table 1**) presenting clinical and morphological features of classic APL. Both *CPSF6* and *RARG* map on chromosome 12. In addition, a *WT1* mutation was identified. The rearrangement within chromosome 12 led to the generation of two different fusion transcripts, depending on the breaking points: the major transcript made up by *CPSF6* exon 4 fused to *RARG* exon 2 and the minor transcript by a deletion of 3 bp at the 5' end of *RARG* exon 2. As regards therapy, the patient was treated with ATRA, but the ATRA treatment was interrupted because of the differentiation syndrome, so the best therapy has still to be established (Qin et al., 2018). Two cases of *CPSF6-RARG* rearrangements were then identified, with different genomic aberrations. In patient no. 5 (**Table 1**) a tetraploidy karyotype in two metaphases and a *DNMT3A* mutation were found; in patient no. 6 (**Table 1**) a *del(12)(p12)* and *WT1* and *K-RAS* mutations were detected. Both patients showed ATRA resistance. The breakpoint in *CPSF6* was located in intron 4 in both patients, while there were 2 breakpoints in intron 3 or 5'UTR and the telomeric region of exon 9 of *RARG*. The 3' region of *RARG* (from exon 1 or exon 4 to exon 9) was reversed and fused in frame with the 5' region of the *CPSF6* gene (from exon 1 to exon 4). Even in these rearrangements, RAR $\gamma$  retained DBD and LBD domain, as well as in the *NUP98-RARG* rearrangements described before. Moreover, the *RARG* breakpoint in patient no. 5 is the same as in *NUP98-RARG*, patient no.1 (**Table 1**) (Such et al., 2011; Liu et al., 2018). These two cases were included in a study which enrolled 1,401 patients with suspected AML. Among these, 19 patients had alternative *RARA* or *RARG* rearrangements. The paper shows an interesting point about the prognostic impact of the presence of this kind of rearrangements: the overall survival and the leukemia-free survival were significantly lower in patients with alternative *RARA* or *RARG* fusions in comparison with patients with the *PML-RARA* classic gene fusion (Wen et al., 2019). Interestingly, the *RARG-CPSF6* fusion was identified in a 26-years-old patient (no. 7, **Table 1**) with AML resembling APL, with a profoundly rearranged region on chromosome 12, where both *RARG* and *CPSF6* are mapped, featuring breakpoints in these two genes and in the eukaryotic translation initiation factor 4B (*EIF4B*). *RARG* intron 9 was fused to *EIF4B* intron 8, but there was a deletion after this point and *EIFB4* was then fused into the intron leading into *CPSF6* exon 6. These rearrangements led to the formation of a novel *RARG-CPSF6* transcript fusion, but there was no evidence of the corresponding protein (Miller et al., 2018).

## NPM1-RARG-NPM1

Recently, another gene involved in the *RARG* rearrangement was identified. *NPM1* is the nucleophosmin 1 gene mapped on

chromosome 5 and it has been indicated as a partner of *RARA* in APL variant translocations (Kikuma et al., 2015). In this AML case (no. 8, **Table 1**), *NPM1* is fused with *RARG*. There was a deletion of about 16 kb of *NPM1* from intron 4 to intron 10 and an insertion of about 23 kb of *RARG* from 5' UTR to intron 9. Sequencing analysis revealed that both *NPM1* and *RARG* had two breakpoints each. The *NPM1* breakpoints were located in intron 4 and intron 10; the *RARG* breakpoints were identified in 5'UTR and intron 9. The presence of three *NPM1-RARG-NPM1* different chimeric transcripts was confirmed: from exon 1 to exon 4 of *NPM1* fused with *RARG*, from partial exon 1 to exon 9, then fused with exon 11 of *NPM1*; exon 1 to exon 4 of *NPM1* fused with *RARG*, from exon 2 to exon 9, then fused with exon 11 of *NPM1*; exon 1 to exon 4 of *NPM1* fused with *RARG*, from exon 4 to exon 9, then fused with exon 11 of *NPM1*. In all three transcripts *RARG* maintained its DBD, while the deletion of *RARG* exon 10 resulted in the loss of LBD 25 amino acids. The patient, like others harboring *RARG* rearrangements, had an APL-like clinical presentation and morphological features, so he received ATRA treatment, but resulted resistant. This is a unique case of AML with *NPM1-RARG-NPM1* chimeric fusion (Chen et al., 2019).

## PML-RARG

*PML* is well-known to be involved in translocation with *RARA* in APL, in which they produce the *PML-RAR $\alpha$*  chimeric protein that behaves as an altered *RAR $\alpha$* , and represses the transcription of RA target genes (Kakizuka et al., 1991). *In vitro* and *in vivo* studies had already demonstrated that *PML-RAR $\gamma$*  has an oncogenic potential (Marinelli et al., 2007, 2009). Ha et al. identified a novel involvement of *PML* in a fusion with *RARG* in an AML patient (no. 9, **Table 1**) with morphologic and immunophenotypic features of classical APL. The translocation *t(12;15)(q13;q22)* was identified; the breakpoints were on intron 3 of *PML* and the 5' upstream region of *RARG*. Two kinds of *PML-RARG* transcripts arise from this gene fusion: a longer transcript (*PML* exon3-*RARG* exon1 middle point) and a shorter one (*PML* exon3-*RARG* exon2). RAR $\gamma$  preserves its DBD and LBD, as in the other cases. It may be interesting to note that the fusion site of *PML* in this rearrangement is consistent with breakpoint cluster region 3 (bcr3) type of *PML-RARA*. The patient had intermittent ATRA treatment, so the sensitivity was not clarified (Ha et al., 2017).

## RARG Epigenetic Alteration

Physiologically, the enhancer of zeste 2 polycomb repressive complex 2 subunit (*EZH2*) is involved in gene silencing thanks to its capability to recruit DNA methyltransferases (DNMTs) for gene repression and its interaction with two epigenetic repression systems (Viré et al., 2006; Sashida and Iwama, 2017). Epigenetic alterations of *RARs* genes can also lead to an AML resembling APL. In one patient (no. 10, **Table 1**) presenting similar features of APL an *EZH2* mutation was found by our group, without any *RARs* rearrangement (Coccaro et al., 2018). The *EZH2* mutation was in its DNMT binding region. Analysis showed a profound downregulation of *RARA* and *RARG* expression compared with APL, AML and normal control pools (Coccaro et al., 2018).

Zhang et al. also reported that a patient harboring *ELL-MLL* (no. 11, **Table 1**) can seem to have APL; in particular *RARG* was upregulated (Zhang et al., 2019a).

## CONCLUSIONS

Despite the diversity of gene partners, *RARG* rearrangements identified up to now have shown similar features, such as the retention of DBD and LBD RAR $\gamma$  domains; the presence of the chimeric transcript, but not always of the protein, and ATRA resistance. Probably, DBD/LBD maintenance affects the fusion protein different nuclear localization and is involved in the leukemogenesis process, leading to an alteration especially of the granulocyte lineage, as well as *PML-RARA*. Nevertheless, RAR $\gamma$ , like RAR $\alpha$ , is critical for the physiological hemopoietic process, although its role is not comparable. In fact, RAR $\gamma$  is involved in maintaining a balance between the self-renewal and differentiation of HSCs rather than in the granulocytic differentiation. Surely, RAR $\gamma$  exhibits an indirect role in this, but it is not clear why *RARG* rearrangements are capable of resembling APL phenotype. Hence, this aspect must be more closely investigated, mainly because these patients seem to be

ATRA-resistant. Indeed, ATRA responsivity also needs to be clarified since *in vitro* and *in vivo* studies have shown contrasting results. In addition, in most reported cases, several mutations in different genes have been identified, especially in the *WT1* gene. This factor could be crucial in the oncogenic process and might also influence ATRA sensitivity. In our opinion, further studies are needed to better understand the oncogenic role of *RARG* rearrangements, and the molecular features of both the chimeric transcript and fusion protein, in particular to define the best therapy for these APL atypical patients.

## AUTHOR CONTRIBUTIONS

MC and IR conceived and wrote the review. LA and AZ performed the literature analysis. FA and GS supervised and approved the final manuscript.

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## REFERENCES

- Al Tanoury, Z., Piskunov, A., and Rochette-Egly, C. (2013). Vitamin A and retinoid signaling: genomic and nongenomic effects. *J. Lipid Res.* 54, 1761–1775. doi: 10.1194/jlr.R038033
- Arber, D. A., Orazi, A., Hasserjian, R., Thiele, J., Borowitz, M. J., Le Beau, M. M., et al. (2016). The 2016 revision to the World Health Organization classification of myeloid neoplasms and acute leukemia. *Blood* 127, 2391–2405. doi: 10.1182/blood-2016-03-643544
- Asou, N., Adachi, K., Tamura, J., Kanamaru, A., Kageyama, S., Hiraoka, A., et al. (1998). Analysis of prognostic factors in newly diagnosed acute promyelocytic leukemia treated with all-trans retinoic acid and chemotherapy. Japan Adult Leukemia Study Group. *J. Clin. Oncol.* 16, 78–85. doi: 10.1200/JCO.1998.16.1.78
- Bastien, J., and Rochette-Egly, C. (2004). Nuclear retinoid receptors and the transcription of retinoid-target genes. *Gene* 328, 1–16. doi: 10.1016/j.gene.2003.12.005
- Beard, R. L., Duong, T. T., Teng, M., Klein, E. S., Standevan, A. M., and Chandraratna, R. A. S. (2002). Synthesis and biological activity of retinoic acid receptor- $\alpha$  specific amides. *Bioorg. Med. Chem. Lett.* 12, 3145–3148. doi: 10.1016/S0960-894X(02)00647-9
- Binothman, N., Hachim, I. Y., Lebrun, J.-J., and Ali, S. (2017). CPSF6 is a clinically relevant breast cancer vulnerability target: role of CPSF6 in breast cancer. *EBioMedicine* 21, 65–78. doi: 10.1016/j.ebiom.2017.06.023
- Blumberg, B., Mangelsdorf, D. J., Dyck, J. A., Bittner, D. A., Evans, R. M., and De Robertis, E. M. (1992). Multiple retinoid-responsive receptors in a single cell: families of retinoid 'X' receptors and retinoic acid receptors in the *Xenopus* egg. *Proc. Natl. Acad. Sci. U.S.A.* 89, 2321–2325. doi: 10.1073/pnas.89.6.2321
- Chambon, P. (1993). The molecular and genetic dissection of the retinoid signalling pathway. *Gene* 135, 223–228. doi: 10.1016/0378-1119(93)90069-F
- Chambon, P. (1996). A decade of molecular biology of retinoic acid receptors. *FASEB J.* 10, 940–954. doi: 10.1096/FASEBJ.10.9.8801176
- Chen, C. F., Goyette, P., and Lohnes, D. (2004). RAR $\gamma$  acts as a tumor suppressor in mouse keratinocytes. *Oncogene* 23, 5350–5359. doi: 10.1038/sj.onc.1207682
- Chen, X., Wang, F., Zhang, Y., Teng, W., Cao, P., Ma, X., et al. (2019). A novel NPM1-RARG-NPM1 chimeric fusion in acute myeloid leukaemia resembling acute promyelocytic leukaemia but resistant to all-trans retinoic acid and arsenic trioxide. *Br. J. Cancer* 120, 1023–1025. doi: 10.1038/s41416-019-0456-z
- Choudhry, A., and DeLoughery, T. G. (2012). Bleeding and thrombosis in acute promyelocytic leukemia. *Am. J. Hematol.* 87, 596–603. doi: 10.1002/ajh.23158
- Coccaro, N., Zagaria, A., Orsini, P., Anelli, L., Tota, G., Casieri, P., et al. (2018). RARA and RARG gene downregulation associated with EZH2 mutation in acute promyelocytic-like morphology leukemia. *Hum. Pathol.* 80, 82–86. doi: 10.1016/j.humpath.2018.02.023
- Conserva, M. R., Anelli, L., Zagaria, A., Specchia, G., and Albano, F. (2019). The pleiotropic role of retinoic acid/retinoic acid receptors signaling: from vitamin A metabolism to gene rearrangements in acute promyelocytic leukemia. *Int. J. Mol. Sci.* 20:2921. doi: 10.3390/ijms20122921
- Daver, N., Kantarjian, H., Marcucci, G., Pierce, S., Brandt, M., Dinardo, C., et al. (2015). Clinical characteristics and outcomes in patients with acute promyelocytic leukaemia and hyperleucocytosis. *Br. J. Haematol.* 168, 646–653. doi: 10.1111/bjh.13189
- De Kouchkovsky, I., and Abdul-Hay, M. (2016). Acute myeloid leukemia: a comprehensive review and 2016 update. *Blood Cancer J.* 6:e441. doi: 10.1038/bcj.2016.50
- de Thé, H., Lavau, C., Marchio, A., Chomienne, C., Degos, L., and Dejean, A. (1991). The PML-RAR $\alpha$  fusion mRNA generated by the t(15;17) translocation in acute promyelocytic leukemia encodes a functionally altered RAR. *Cell* 66, 675–684. doi: 10.1016/0092-8674(91)90113-D
- Dewamitta, S. R., Joseph, C., Purton, L. E., and Walkley, C. R. (2014). Erythroid-extrinsic regulation of normal erythropoiesis by retinoic acid receptors. *Br. J. Haematol.* 164, 280–285. doi: 10.1111/bjh.12578
- Ellinger-Ziegelbauer, H., and Dreyer, C. (1991). A retinoic acid receptor expressed in the early development of *Xenopus laevis*. *Genes Dev.* 5, 94–104. doi: 10.1101/gad.5.1.94
- Giguère, V., Ong, E. S., Evans, R. M., and Tabin, C. J. (1989). Spatial and temporal expression of the retinoic acid receptor in the regenerating amphibian limb. *Nature* 337, 566–569. doi: 10.1038/337566a0
- Green, A. C., Poulton, I. J., Vrahnas, C., Häusler, K. D., Walkley, C. R., Wu, J. Y., et al. (2015). RAR $\gamma$  is a negative regulator of osteoclastogenesis. *J. Steroid Biochem. Mol. Biol.* 150, 46–53. doi: 10.1016/j.jsbmb.2015.03.005
- Green, A. C., Rudolph-Stringer, V., Straszkowski, L., Tjin, G., Crimée-irwin, B., Walia, M., et al. (2018). Retinoic acid receptor  $\gamma$  activity in mesenchymal stem

- cells regulates endochondral bone, angiogenesis, and B lymphopoiesis. *J. Bone Miner. Res.* 33, 2202–2213. doi: 10.1002/jbmr.3558
- Ha, J.-S., Do, Y. R., Ki, C.-S., Lee, C., Kim, D.-H., Lee, W., et al. (2017). Identification of a novel PML-RARG fusion in acute promyelocytic leukemia. *Leukemia* 31, 1992–1995. doi: 10.1038/leu.2017.167
- Ishikawa, T., Umesono, K., Mangelsdorf, D. J., Aburatani, H., Stanger, B. Z., Shibasaki, Y., et al. (1990). A functional retinoic acid receptor encoded by the gene on human chromosome 12. *Mol. Endocrinol.* 4, 837–844. doi: 10.1210/mend-4-6-837
- Joseph, C., Nota, C., Fletcher, J. L., Maluenda, A. C., Green, A. C., and Purton, L. E. (2016). Retinoic acid receptor  $\gamma$  regulates B and T lymphopoiesis via nestin-expressing cells in the bone marrow and thymic microenvironments. *J. Immunol.* 196, 2132–2144. doi: 10.4049/jimmunol.1501246
- Kakizuka, A., Miller, W. H., Umesono, K., Warrell, R. P., Frankel, S. R., Murty, V. V., et al. (1991). Chromosomal translocation  $t(15;17)$  in human acute promyelocytic leukemia fuses RAR alpha with a novel putative transcription factor, PML. *Cell* 66, 663–74.
- Karim, F., Shaikh, U., Adil, S. N., and Khurshid, M. (2014). Clinical characteristics, outcome and early induction deaths in patients with acute promyelocytic leukaemia: a five-year experience at a tertiary care centre. *Singapore Med. J.* 55, 443–447. doi: 10.11622/SMEDJ.2014105
- Kashyap, V., Laursen, K. B., Brenet, F., Viale, A. J., Scandura, J. M., and Gudas, L. J. (2013). RAR $\gamma$  is essential for retinoic acid induced chromatin remodeling and transcriptional activation in embryonic stem cells. *J. Cell Sci.* 126, 999–1008. doi: 10.1242/jcs.119701
- Kastner, P., Krust, A., Mendelsohn, C., Garnier, J. M., Zelent, A., Leroy, P., et al. (1990). Murine isoforms of retinoic acid receptor gamma with specific patterns of expression. *Proc. Natl. Acad. Sci. U.S.A.* 87, 2700–4. doi: 10.1073/pnas.87.7.2700
- Kastner, P., Leid, M., and Chambon, P. (1994). “Metabolism of vitamin A, the retinoid-binding protein superfamily,” in *Vitamin A in Health and Disease*, ed. R. Blomhoff Dekker (New York, NY: Rune Blomhoff) 87–118.
- Kikuma, T., Nakamachi, Y., Noguchi, Y., Okazaki, Y., Shimomura, D., Yakushijin, K., et al. (2015). A new transcriptional variant and small azurophilic granules in an acute promyelocytic leukemia case with NPM1/RARA fusion gene. *Int. J. Hematol.* 102, 713–718. doi: 10.1007/s12185-015-1857-2
- La Starza, R., Brandimarte, L., Pierini, V., Nofrini, V., Gorello, P., Crescenzi, B., et al. (2009). A NUP98-positive acute myeloid leukemia with a  $t(11;12)(p15;q13)$  without HOXC cluster gene involvement. *Cancer Genet. Cytogenet.* 193, 109–111. doi: 10.1016/j.cancergencyto.2009.04.015
- Leroy, P., Krust, A., Zelent, A., Mendelsohn, C., Garnier, J. M., Kastner, P., et al. (1991). Multiple isoforms of the mouse retinoic acid receptor alpha are generated by alternative splicing and differential induction by retinoic acid. *EMBO J.* 10, 59–69. doi: 10.1002/j.1460-2075.1991.tb07921.x
- Levine, R. L. (2013). Molecular pathogenesis of AML: Translating insights to the clinic. *Best Pract. Res. Clin. Haematol.* 26, 245–248. doi: 10.1016/j.BEHA.2013.10.003
- Liu, Q., and Linney, E. (1993). The mouse retinoid-X receptor-gamma gene: genomic organization and evidence for functional isoforms. *Mol. Endocrinol.* 7, 651–658. doi: 10.1210/mend.7.5.8391126
- Liu, T., Wen, L., Yuan, H., Wang, Y., Yao, L., Xu, Y., et al. (2018). Identification of novel recurrent CPSF6-RARG fusions in acute myeloid leukemia resembling acute promyelocytic leukemia. *Blood* 131, 1870–1873. doi: 10.1182/blood-2017-11-818716
- Lohnes, D., Kastner, P., Dierich, A., Mark, M., LeMeur, M., and Chambon, P. (1993). Function of retinoic acid receptor  $\gamma$  in the mouse. *Cell* 73, 643–658. doi: 10.1016/0092-8674(93)90246-M
- Luo, H., Zhang, S., Li, K., Chen, X.-H., Li, Y.-C., Sun, Y., et al. (2019). A novel entity of acute myeloid leukaemia with recurrent RARG-rearrangement resembling acute promyelocytic leukaemia. *Leuk. Res.* 77, 14–16. doi: 10.1016/j.leukres.2018.12.009
- Marinelli, A., Bossi, D., Pelicci, P. G., and Minucci, S. (2007). A redundant oncogenic potential of the retinoic receptor (RAR)  $\alpha$ ,  $\beta$  and  $\gamma$  isoforms in acute promyelocytic leukemia. *Leukemia* 21, 647–650. doi: 10.1038/sj.leu.2404572
- Marinelli, A., Bossi, D., Pelicci, P. G., and Minucci, S. (2009). Redundant function of retinoic acid receptor isoforms in leukemogenesis unravels a prominent function of genome topology and architecture in the selection of mutagenic events in cancer. *Leukemia* 23, 417–419. doi: 10.1038/leu.2008.205
- Mark, M., Ghyselinck, N. B., and Chambon, P. (2006). Function of retinoid nuclear receptors: lessons from genetic and pharmacological dissections of the retinoic acid signaling pathway during mouse embryogenesis. *Annu. Rev. Pharmacol. Toxicol.* 46, 451–480. doi: 10.1146/annurev.pharmtox.46.120604.141156
- Mattei, M. G., Petkovich, M., Mattei, J. F., Brand, N., and Chambon, P. (1988). Mapping of the human retinoic acid receptor to the q21 band of chromosome 17. *Hum. Genet.* 80, 186–188.
- Mattei, M. G., Rivière, M., Krust, A., Ingvarsson, S., Vennström, B., Islam, M. Q., et al. (1991). Chromosomal assignment of retinoic acid receptor (RAR) genes in the human, mouse, and rat genomes. *Genomics* 10, 1061–1069.
- McGowan, S., Jackson, S. K., Jenkins-Moore, M., Dai, H.-H., Chambon, P., and Snyder, J. M. (2000). Mice bearing deletions of retinoic acid receptors demonstrate reduced lung elastin and alveolar numbers. *Am. J. Respir. Cell Mol. Biol.* 23, 162–167. doi: 10.1165/ajrcmb.23.2.3904
- Meister, B., Fink, F. M., Hittmair, A., Marth, C., and Widschwendter, M. (1998). Antiproliferative activity and apoptosis induced by retinoic acid receptor-gamma selectively binding retinoids in neuroblastoma. *Anticancer Res.* 18, 1777–86.
- Mic, F. A., Molotkov, A., Benbrook, D. M., and Duester, G. (2003). Retinoid activation of retinoic acid receptor but not retinoid X receptor is sufficient to rescue lethal defect in retinoic acid synthesis. *Proc. Natl. Acad. Sci. U.S.A.* 100, 7135–7140. doi: 10.1073/pnas.1231422100
- Miller, C. A., Tricarico, C., Skidmore, Z. L., Uy, G. L., Lee, Y.-S., Hassan, A., et al. (2018). A case of acute myeloid leukemia with promyelocytic features characterized by expression of a novel RARG-CPSF6 fusion. *Blood Adv.* 2, 1295–1299. doi: 10.1182/bloodadvances.2017014183
- Millevoi, S., and Vagner, S. (2010). Molecular mechanisms of eukaryotic pre-mRNA 3' end processing regulation. *Nucleic Acids Res.* 38, 2757–2774. doi: 10.1093/nar/gkp1176
- Nagpal, S., Saunders M., Kastner P., Durand B., Nakshatri H., and Chambon, P. (1992). Promoter context- and response element-dependent specificity of the transcriptional activation and modulating functions of retinoic acid receptors. *Cell* 70, 1007–1019.
- Noji, S., Nohno, T., Koyama, E., Muto, K., Ohshima, K., Aoki, Y., et al. (1991). Retinoic acid induces polarizing activity but is unlikely to be a morphogen in the chick limb bud. *Nature* 350, 83–86. doi: 10.1038/350083a0
- Osumi, T., Tsujimoto, S. ichi, Tamura, M., Uchiyama, M., Nakabayashi, K., Okamura, K., et al. (2018). Recurrent RARB translocations in acute promyelocytic leukemia lacking RARA translocation. *Cancer Res.* 78, 4452–4458. doi: 10.1158/0008-5472.CAN-18-0840
- Padanilam, B. J., Mcleod, L. B., Suzuki, H., and Solursh, M. (1991). Nucleotide sequence of an isoform of chicken retinoic acid binding protein- $\beta$  varying in its A domain. *Nucleic Acids Res.* 19, 395–395. doi: 10.1093/nar/19.2.395
- Purton, L. E. (2007). Roles of retinoids and retinoic Acid receptors in the regulation of hematopoietic stem cell self-renewal and differentiation. *PPAR Res.* 2007:87934. doi: 10.1155/2007/87934
- Purton, L. E., Dworkin, S., Olsen, G. H., Walkley, C. R., Fabb, S. A., Collins, S. J., et al. (2006). RARgamma is critical for maintaining a balance between hematopoietic stem cell self-renewal and differentiation. *J. Exp. Med.* 203, 1283–1293. doi: 10.1084/jem.20052105
- Qin, Y.-Z., Huang, X.-J., and Zhu, H.-H. (2018). Identification of a novel CPSF6-RARG fusion transcript in acute myeloid leukemia resembling acute promyelocytic leukemia. *Leukemia* 32, 2285–2287. doi: 10.1038/s41375-018-0095-z
- Qiu, J. J., Zeisig, B. B., Li, S., Liu, W., Chu, H., Song, Y., et al. (2015). Critical role of retinoid/retinoid signaling in mediating transformation and therapeutic response of NUP98-RARG leukemia. *Leukemia* 29, 1153–1162. doi: 10.1038/leu.2014.334
- Ragsdale, C. W., Petkovich, M., Gates, P. B., Chambon, P., and Brockes, J. P. (1989). Identification of a novel retinoic acid receptor in regenerative tissues of the newt. *Nature* 341, 654–657. doi: 10.1038/341654a0
- Rasheedi, S., Shun, M.-C., Serrao, E., Sowd, G. A., Qian, J., Hao, C., et al. (2016). The cleavage and polyadenylation specificity factor 6 (CPSF6) subunit of the capsid-recruited pre-messenger RNA cleavage factor I (CFIm) complex mediates HIV-1 integration into genes. *J. Biol. Chem.* 291, 11809–11819. doi: 10.1074/jbc.M116.721647
- Romana, S., Radford-Weiss, I., Abdelali, R. Ben, Schluth, C., Petit, A., Dastugue, N., et al. (2006). NUP98 rearrangements in hematopoietic malignancies: a study

- of the Groupe Francophone de Cytogénétique Hématologique. *Leukemia* 20, 696–706. doi: 10.1038/sj.leu.2404130
- Rowe, A., Richman, J. M., and Brickell, P. M. (1991). Retinoic acid treatment alters the distribution of retinoic acid receptor-beta transcripts in the embryonic chick face. *Development* 111, 1007–1016.
- Sashida, G., and Iwama, A. (2017). Multifaceted role of the polycomb-group gene EZH2 in hematological malignancies. *Int. J. Hematol.* 105, 23–30. doi: 10.1007/s12185-016-2124-x
- Sill, H., Olipitz, W., Zebisch, A., Schulz, E., and Wölfler, A. (2011). Therapy-related myeloid neoplasms: pathobiology and clinical characteristics. *J. Pharmacol.* 162, 792–805. doi: 10.1111/j.1476-5381.2010.01100.x
- Smith, S. M., and Eichele, G. (1991). Temporal and regional differences in the expression pattern of distinct retinoic acid receptor-beta transcripts in the chick embryo. *Development* 111, 245–252.
- Spanjaard, R. A., Ikeda, M., Lee, P. J., Charpentier, B., Chin, W. W., and Eberlein, T. J. (1997). Specific activation of retinoic acid receptors (RARs) and retinoid X receptors reveals a unique role for RAR $\gamma$  in induction of differentiation and apoptosis of S91 melanoma cells. *J. Biol. Chem.* 272, 18990–18999. doi: 10.1074/jbc.272.30.18990
- Storzazzi, C. T., Albano, F., Lo Cunsolo, C., Doglioni, C., Guastadisegni, M. C., Impera, L., et al. (2007). Upregulation of the SOX5 by promoter swapping with the P2RY8 gene in primary splenic follicular lymphoma. *Leukemia* 21, 2221–2225. doi: 10.1038/sj.leu.2404784
- Such, E., Cervera, J., Valencia, A., Barragán, E., Ibañez, M., Luna, I., et al. (2011). A novel NUP98/RAR $\gamma$  gene fusion in acute myeloid leukemia resembling acute promyelocytic leukemia. *Blood* 117, 242–245. doi: 10.1182/blood-2010-06-291658
- Such, E., Cerdón, L., Sempere, A., Villamón, E., Ibañez, M., Luna, I., et al. (2014). In vitro all-trans retinoic acid sensitivity of acute myeloid leukemia blasts with NUP98/RAR $\gamma$  fusion gene. *Ann. Hematol.* 93, 1931–1933. doi: 10.1007/s00277-014-2073-5
- Szotkowski, T., Faber, E., Hubacek, J., Raida, L., Rohon, P., Kuba, A., et al. (2015). Acute promyelocytic leukemia successfully treated also in elderly patients with significant comorbidities: a 20-year single-center experience. *Neoplasia* 62, 146–151. doi: 10.4149/neo\_2015\_019
- Taneja, R., Bouillet, P., Boylan, J. F., Gaub, M. P., Roy, B., Gudas, L. J., et al. (1995). Reexpression of retinoic acid receptor (RAR) gamma or overexpression of RAR alpha or RAR beta in RAR gamma-null F9 cells reveals a partial functional redundancy between the three RAR types. *Proc. Natl. Acad. Sci. U.S.A.* 92, 7854–7858. doi: 10.1073/pnas.92.17.7854
- Thomas, D., and Majeti, R. (2017). Biology and relevance of human acute myeloid leukemia stem cells. *Blood* 129, 1577–1585. doi: 10.1182/blood-2016-10-696054
- Viré, E., Brenner, C., Deplus, R., Blanchon, L., Fraga, M., Didelot, C., et al. (2006). The Polycomb group protein EZH2 directly controls DNA methylation. *Nature* 439, 871–874. doi: 10.1038/nature04431
- Walkley, C. R., Olsen, G. H., Dworkin, S., Fabb, S. A., Swann, J., McArthur, G. A., et al. (2007). A microenvironment-induced myeloproliferative syndrome caused by retinoic acid receptor gamma deficiency. *Cell* 129, 1097–1110. doi: 10.1016/j.cell.2007.05.014
- Wen, L., Xu, Y., Yao, L., Wang, N., Wang, Q., Liu, T., et al. (2019). Clinical and molecular features of acute promyelocytic leukemia with variant retinoic acid receptor fusions. *Haematologica* 104, e195–e199. doi: 10.3324/haematol.2018.205369
- Wendling, O., Ghyselinck, N. B., Chambon, P., and Mark, M. (2001). Roles of retinoic acid receptors in early embryonic morphogenesis and hindbrain patterning. *Development* 128, 2031–2038.
- Williams, J. A., Kondo, N., Okabe, T., Takeshita, N., Pilchak, D. M., Koyama, E., et al. (2009). Retinoic acid receptors are required for skeletal growth, matrix homeostasis and growth plate function in postnatal mouse. *Dev. Biol.* 328, 315–327. doi: 10.1016/j.ydbio.2009.01.031
- Zelent, A., Mendelsohn, C., Kastner, P., Krust, A., Garnier, J. M., Ruffenach, F., et al. (1991). Differentially expressed isoforms of the mouse retinoic acid receptor beta generated by usage of two promoters and alternative splicing. *EMBO J.* 10, 71–81. doi: 10.1002/j.1460-2075.1991.tb07922.x
- Zhang, X., Huang, X., Xu, H., Li, J., and Yu, W. (2019a). MLL-rearrangement can resemble acute promyelocytic leukemia. *Leuk. Lymphoma* 1–3. doi: 10.1080/10428194.2019.1607328
- Zhang, X., Li, F., Wang, J., Suo, S., Ling, Q., Yu, W., et al. (2019b). RAR $\gamma$ -rearrangements resemble acute promyelocytic leukemia and benefit from 3 + 7 regimen. *Leuk. Lymphoma* 60, 1831–1834. doi: 10.1080/10428194.2018.1553302

**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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