Chemotherapy resistance mechanisms in advanced skin cancer

Bhuvanesh Sukhlal Kalal,[1,2] Dinesh Upadhya,[2] Vinitha Ramanath Pai[1]

[1]Department of Biochemistry, Yenepoya Medical College; [2]Yenepoya Research Centre, Yenepoya University, Mangaluru, India

Abstract

Melanoma is a most dangerous and deadly type of skin cancer, and considered intrinsically resistant to both radiotherapy and chemotherapy. It has become a major public health concern as the incidence of melanoma has been rising steadily over recent decades with a 5-year survival remaining less than 5%. Detection of the disease in early stage may be curable, but late stage metastatic disease that has spread to other organs has an extremely poor prognosis with a median survival of less than 10 months. Since metastatic melanoma is unresponsive to therapy that is currently available, research is now focused on different treatment strategies such as combinations of surgery, chemotherapy and radiotherapy. The molecular basis of resistance to chemotherapy seen in melanoma is multifactorial; defective drug transport system, altered apoptotic pathway, deregulation of apoptosis and/or changes in enzymatic systems that mediate cellular metabolic machinery. Understanding of alterations in molecular processes involved in drug resistance may help in developing new therapeutic approaches to treatment of malignant melanoma.

Correspondence: Bhuvanesh Sukhlal Kalal, Department of Biochemistry, Yenepoya Medical College, Yenepoya University, Mangaluru -575018, Karnataka, India; and Yenepoya Research Centre, Yenepoya University, Mangaluru -575018, Karnataka, India.
Tel.: +91.824.2204668 (Ext: 2017) | +91.9739309942.
E-mail: bhuvanesh611@gmail.com

Key words: Drug-resistance; melanoma; chemotherapy; *BRAF* mutation; combination therapy; review.

Acknowledgments: we thank the Board of Research in Nuclear Sciences (BRNS), Department of Atomic Energy, Government of India for the financial support (Sanction no: 2013/34/8; sanctioned to DU) and Yenepoya University for Junior Research Fellowship to BSK.

Contributions: BSK wrote the manuscript and prepared the figure and the table. DU and VRP reviewed the manuscript. All authors read and approved the final manuscript.

Conflict of interest: there are no conflicts of interest.

Received for publication: 16 November 2016.
Revision received: 24 February 2017.
Accepted for publication: 27 February 2017.

Oncology Reviews 2017; 11:326
doi:10.4081/oncol.2017.326

Introduction

The development of resistance to chemotherapy continues to be the major impediment in the treatment of cancer patients. Newer agents, whether chemotherapeutic or targeted, are constantly being developed. Though most anticancer therapies will alter tumor growth, in most cases the effect is not long lasting and treatment failure has an impact on the survival of advanced skin cancer patients. Consequently, there is a significant need for newer agents with low susceptibility to common drug resistance mechanisms in order to improve response rates and potentially extend survival.[1]

Melanoma is a rare form of skin cancer, which develops through the malignant transformation of melanocytes. It has the fastest growing incidence of any cancer among men and the second fastest growing incidence in women.[2] If recognized and treated early, most cases of melanoma are curable. However, once metastasized it is difficult to treat and is responsible for 80% of deaths related to skin cancers.[3,4] The efficiency of treatments for metastatic melanoma has not significantly improved over the past 50 years with the 5-year survival rate being less than 5%.[5-7] The American Cancer Society estimated that 9,730 deaths will occur due to melanoma in 2017 with an incidence of 3.1/100,000 in USA, as well as 3,400 deaths from other forms of skin cancer, excluding basal cell and squamous cell carcinomas.[8] The World Health Organization has estimated that more than 65,000 people die from melanoma in a year worldwide.[9] Surgery, radiotherapy and chemotherapies are the commonly used therapies for non-metastasized melanoma. However, once metastasized, the treatment options for melanoma become very limited.

Chemotherapy is an extremely ineffective and unsatisfactory means of treating malignant melanoma due to drug resistance, which is characteristic of this disease. Development of drug resistance, either by intrinsic at onset (primary or intrinsic resistance) or during application of cytostatic drug (acquired resistance), is a major problem that limits the effectiveness of chemotherapies used to treat malignant melanoma. Therefore, if drug resistance could be overcome, there may be a significant impact on the survival rate. One of the various factors contributing to sensitivity of drugs is the limited amount of drug reaching the tumour and affecting the tumour microenvironment.

In this review, the relevance of certain factors, such as transport mechanisms, various enzyme systems, alterations in drug target, processing of drug-induced damage; and regulatory aspects of apoptosis for melanoma drug resistance, has been discussed.

Molecular mechanisms of drug resistance

The mechanisms conferring this intrinsic drug resistance in melanoma cells are poorly understood. Grottke *et al.* found eleven genes that were differentially expressed upon acquisition of etoposide resistance in malignant melanoma cells. However, most of them were of unknown function and are yet to be characterized. Multiple mechanisms identified in chemoresistance of other tumor cell types have been investigated in melanoma as well[10] (Figure 1).

Drug transport and efflux pump mediated resistance

Drug efflux system is the most commonly observed mechanisms, responsible for reduced intracellular accumulation of cytostatic drugs in drug resistant cell lines. Drug resistance is mediated by ATP-binding cassette (ABC) transporters which actively transport drugs out of the cells.[11] The two classes of ATP-dependent drug transporter proteins, P-glycoprotein (Pgp) and the multidrug resistance-associated proteins (MRPs), mediate drug efflux which reduces drug accumulation and makes tumor cells resistant to the cytotoxic effects of many anticancer agents. Among the 48 known human ABC transporters, melanoma cells express the most studied and targeted mediators of drug resistance such as ABCB1 (MDR1), ABCC1 (MRP1), ABCC2 (MRP2), ABCA9, ABCB5, ABCB8, ABCD1 and ABCG2 (MXR).[12-16] Both ABCB5 and ABCB8 expression are known to facilitate doxorubicin resistance in melanoma cells[13,17] and ABCC2 to mediate cisplatin resistance.[18] Luo *et al.* isolated a subpopulation of drug effluxing cells directly from melanoma patients and named them as 'side population'. These cells were reported to have increased efflux capacity and resistant to paclitaxel by up-regulation of ABCB1 and ABCB5.[15] However, most melanoma cell lines as well as primary and metastatic tumors do not express Pgp and therefore it cannot be regarded as a major common feature mediating drug resistance in human melanoma cells.[19,20] On the other hand, MRP is frequently expressed in melanoma but its expression is not up regulated significantly in response to chemotherapy. Although all drug transporters have not been studied, the present literature is suggestive that induction of drug transporter may not be the prime cause of drug-mediated resistance in skin cancer. Some studies have demonstrated association of melanoma with MRP1[21] and MRP2[22] expression to cytostatic drugs. In a phase I clinical study, only partial response was observed in a patient with melanoma who was treated with epirubicin (MRP1 inhibitor) in combination with sulindac (a non-steroidal anti-inflammatory drug).[23] Therefore, the role of MRP-dependent transport mechanisms in melanoma drug resistance remains unclear.

Drug resistance mediated by altered enzyme activation

Inactivation of drugs can diminish the availability of free drug to bind to its intracellular targets. The intracellular detoxification of many anticancer drugs (mostly alkylating agents) occurs by conjugation with glutathione (GSH), catalyzed by enzyme glutathione-S-transferase (GST).[24-29] GSH is a powerful antioxidant, which inhibits oxidative stress that can damage DNA and RNA. The level of GST was found to be higher in melanoma lesions compared to benign melanocytic nevi.[30,31] The conjugation anticancer drugs with GSH inhibits the conversion of mono-adducts to cross-links, thereby reducing the cytotoxic potential of the adducts. The c-Myc-induced apoptosis was reported to have dependency on the intracellular GSH content in melanoma.[32] However, in a panel

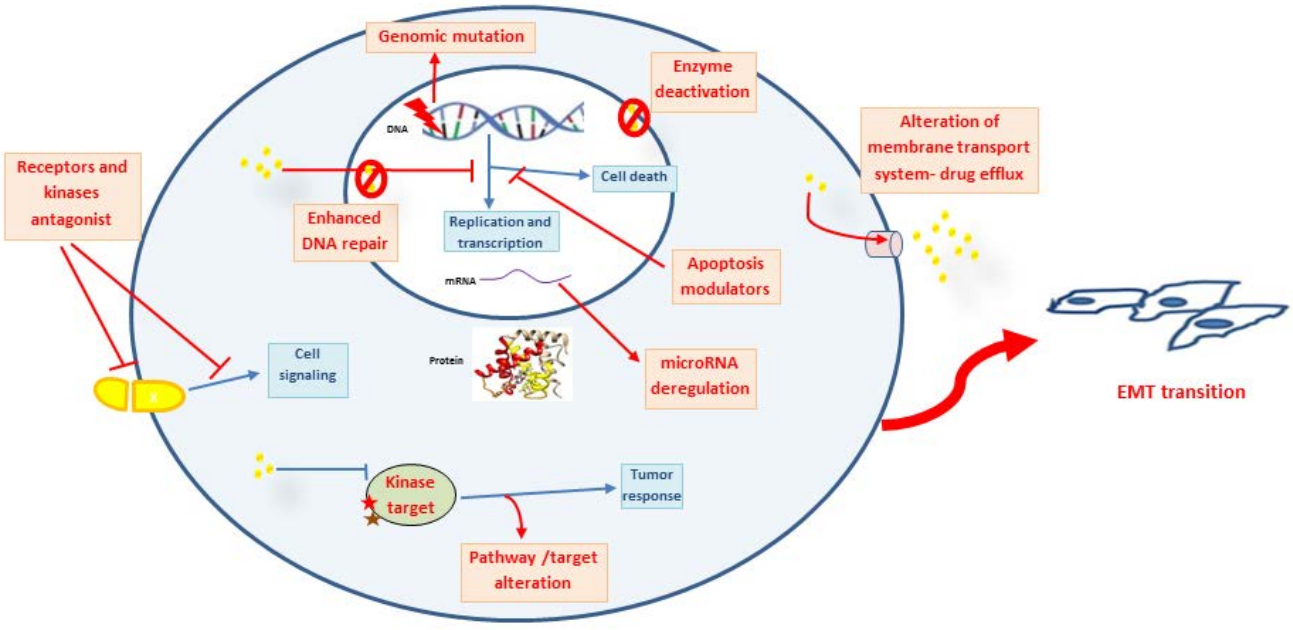

Figure 1. Overview of drug resistance mechanism in melanoma.

of melanomas, no significant correlation was observed between alteration in GST/GSH-metabolism with the course of tumor progression, treatment and clinical response.[26,33,34] In addition, there was neither any increase in GSH concentration in cisplatin cytotoxicity nor was the drug level affected in melanoma cells.[35]

Topoisomerase (Topo) is a nuclear enzyme that plays important roles for DNA transcription and recombination as well as segregation of chromatids during mitosis. It has been targeted by various inhibitory chemotherapeutic agents such as camptothecin and its derivatives, which inhibit Topo I, and doxorubicin, etoposides, mitoxantrone which inhibit Topo II.[36,37] In melanoma, etoposide resistance has been associated both with mutation or deletion[38] and increased activity of Topo II.[39] However, Satherley *et al.* could not establish an association of chemosensitivity with expression of Topo II in choroidal melanoma.[40] Moreover, nitric oxide (NO) oxidation is shown to detoxify etoposide through direct nitrogen oxide radical attack.[41]

Drug resistance mediated by altered DNA repair

The alkylating agents induce cytotoxic O^6-chloroethylguanine DNA lesions *via* adducts formation, followed by inter-strand DNA crosslinks and subsequent inhibition of DNA replication or RNA transcription, leading to arrest of the cell cycle in G2 phase. The DNA repair enzyme O^6-alkylguanine DNA alkyltransferase helps in repair of these adducts thus impairing the cytotoxic effect and mediating a major resistance pathway for these drugs.[42] Drug-resistant melanoma cell lines exhibit increased (base excision) repair of DNA damage.[43] In addition, decreased nuclear mismatch-repair protein expression was associated with a fotemustine-resistant phenotype in drug-resistant melanoma cell lines.[39] Up-regulation associated with increased activity of O^6-methylguanine-DNA methyltransferase (MGMT), an enzyme involved in repair of DNA damage caused by alkylation, was found in melanoma.[44] Clinical trials proving the association of drug response and MGMT expression are inconclusive.[45,46] However, in a recently performed retrospective study no significant correlation of mismatch repair (MMR) expression, alone, or in combination with MGMT levels (with clinical response to dacarbazine-based chemotherapy) could be found,[47] suggesting that these molecules are not predictive of clinical response.

Drug resistance via modulation of the apoptotic pathway

Apoptosis represents a complex genetic program consisting of several pathways. Presently there are two well-characterized caspase-activating cascades that regulate caspase-mediated cell death. The extrinsic pathway is triggered by binding of ligands to their individual cell-surface death receptor (*e.g.*, cluster of differentiation 95 (CD95), tumor necrosis factor-related apoptosis-inducing ligand receptor 1-4 (TRAIL-R1-4), tumor necrosis factor receptor 1(TNF-R1)) which, after oligomerization, recruits adapter molecules and initiator caspases (caspase-8/ caspase-10) that result in a proteolytic cascade.[48,49] Interference of CD95 (Fas/APO-1) mediated apoptosis terminate immune response.[50,51] The intrinsic pathway involves mitochondrial release of cytochrome c that binds to apoptotic protease activating factor-1 (Apaf-1) and thereby induces conformational changes of this apoptotic protein followed by recruitment of procaspase-9 to the complex.[52] Procaspase-9 is subsequently autocatalyzed.[48,49,52] These two pathways converge with the activation of effector caspases, induction of specific endonucleases resulting in DNA fragmentation and cleavage of nuclear proteins essential for nuclear and cellular structure, DNA repair and replication.[53] Cell death or survival is balanced by a number of regulator molecules at multiple levels such as p53, rat sarcoma (Ras), Bcl-2-family proteins or members of the IAP family. Recently, Koetz-Ploch *et al.* showed that in drug-resistant melanoma cells, microRNA-125a promotes the resistance to BRAF (v-raf murine sarcoma viral oncogene homolog B) inhibitors by apoptotic suppression, as well as reactivation of signalling pathways such as mitogen-activated protein kinase (MAPK) and AKT8 virus oncogene cellular homolog (AKT).[54]

p53

Either overexpression of human double minute 2 (Hdm2), a negative regulator that binds and ubiquitinates p53, or loss of the cyclin-dependent kinase inhibitor 2A (CDKN2A) gene encoding alternate reading frame protein (p14ARF), a factor that inhibits the Hdm2-dependent degradation of p53, is commonly observed in metastatic melanoma cells.[49,55,56] In addition, in malignant melanomas, the anti-apoptotic protein Bcl-2 is overexpressed while Apaf-1, an important factor for apoptosome formation in the intrinsic apoptosis pathway, is downregulated.[57-59] Similarly overexpression of induced myeloid leukemia cell differentiation protein (Mcl-1) was observed in drug resistant cell lines.[60] Naumann *et al.* showed that the melanoma cell lines expressing p53 wild-type were more resistant to temozolomide (TMZ) and fotemustine than p53 mutant melanoma lines, which suggest that the role of p53 in the regulation of apoptosis upon TMZ treatment could be different in melanoma cells than in other cancer cells.[61] Melanoma treated with DNA cross-linking drugs acquire resistance by p53-dependent up-regulation of DNA repair genes, xeroderma pigmentosum complementation group C (XPC) and damaged DNA-binding protein 2 (DDB2).[62]

The BRAF-mutated melanoma develops resistance to RAF inhibitors through several genetic changes that leads to the reactivation of the MAPK.[63,64] The widely observed mechanisms are mutation of neuroblastoma-RAS (NRAS mutations-$NRAS^{Q61}$; $NRAS^{T58}$; $NRAS^{G13R}$),[65] loss of neurofibromatosis type-1(NF1),[66-68] loss of phosphatase and tensin homolog (PTEN),[69,70] up-regulation of cancer Osaka thyroid kinase (COT),[71] mutation of MEK,[63,69] negative feedback inactivation of extracellular-related kinase (ERK) and alteration in phosphoinositide 3-kinase-AKT (PI3K-AKT) pathway.[64,72]

Approaches to overcoming drug resistance

The combination of targeted therapies and immunotherapy has the potential for synergism via variety of mechanisms.[73] By blocking an oncogenic mutation, targeted therapies may trigger tumor cell senescence and allow tumor clearance by T-cells.[74] The concurrent release of antigenic debris from tumor cell death may also contribute to the success of immunotherapy by maximizing dendritic cell activation. Lastly, the induction of tumor regression by targeted therapies and the potential decrease in tumor associated immunosuppression, may enable a more favorable environment for immunotherapy to have an effect.[73] Promising agents that target programmed death (PD1) have also emerged[75] and one such ligands is PD- L17.[76] PD-1 protein, a T-cell coinhibitory receptor, and the ligand PD-L1 play a key role in the ability of tumor cells

Table 1. Mechanisms of resistance to anticancer drugs in melanoma.

Resistance mechanism	Examples	Reference
Overexpression of drug efflux proteins	Over-expression of Heat Shock Transcription Factor 1 (HSF1) Up-regulation of ABCB1 and ABCB5	78 13,17
Alteration of enzyme activation	Increase in Glutathione (GSH) levels	30-32,34,35
Deregulation of apoptosis	Up-regulation of miR-125a expression P53-dependent up-regulation of XPC and DDB Over-expression of the MCL1 anti-apoptotic BCL-2 family member Down-regulation of Apaf-1 Down-regulation, loss and mutation of CD95/Fas-receptor	54 62 57,59,60 58 50,51
Ras mutation	$NRAS^{Q61K/R}$ mutations ($NRAS^{Q61}$, NRAST58, $NRAS^{G13R}$) Loss of NF1 COT up-regulation MEK mutation ($MEK1^{C121S}$, $MEK1^{Q56P}$, $MEK1^{K57E}$, $MEK1^{E203K}$, $MEK1^{V60E}$, $MEK1^{G128V}$ and $MEK2^{F57C}$, $MEK2^{C125S}$, $MEK2^{V35M}$, $MEK2^{L46F}$, $MEK2^{N126D}$) CRAF up-regulation, BRAF amplifications, BRAF truncation ERK- negative feedback inactivation Loss of PTEN PI3K-AKT pathway alteration (AKT1/3 ^{Q79K}, AKT1/3 ^{E17K}, mutation in PIK3R2, PHLPP1, PIK3CA and PIK3CG) MITF amplification and feedback suppression	65 66-68 71 63,64,69 79 80 69,70,81 64,72 82-84
Epithelial to mesenchymal transition	Up-regulation of IGF-IR signaling	85
Deregulation of microRNAs expression	Overexpression miR-34a, miR-100, miR-125b miR-195, miR-638, miRNA-1246	86-90

to evade the host immune system. Blockade of PD-1 and PD-L1 has been incontestable to mediate antitumor activity in preclinical models and early clinical evidence has emerged supporting this. In the phase I studies, anti-PD-1 (BMS-936558, nivolumab) and anti-PD-Ll (BMS-936559) antibodies demonstrated response rates of 28 and 17%, respectively in metastatic melanoma patients. A phase Ib study of the anti-PD-Ll antibody, MPDL3280A, with vemurafenib is already underway in treatment-naive patients with BRAF V600-mutant metastatic melanoma (ClinicalTrials.gov; 2012. http://www.clinicaltrials.gov (Clinical-Trials.gov Identifier NCT01656642, NCT01611675, NCT01585 415, NCT01683188, NCT01603212, NCT01659151)). Other studies using drug combination that are designed to exploit the potential benefits of selective BRAF inhibition with immune modulation, include the combinations of vemurafenib with leflunomide, white blood cell therapy, high-dose interleukin-2 (IL-2), interferon and IL-2, or lymphodepletion plus adoptive cell transfer (ClinicalTrials.gov; 2012. http://www.clinicaltrials.gov (Clinical- Trials.gov Identifier NCT01656642, NCT01611675, NCT01585 415, NCT01683188, NCT01603212, NCT01659151)). Recently deregulation of Janus kinase 1 (JAK1) was identified in melanoma resistant to BRAF inhibitors, and to overcome such resistance the use of combination therapy was suggested, which may improve the durability of the response to BRAF inhibitors.[77]

Conclusions

Over the past decade a variety of chemotherapeutic agents have evolved to rechallenge melanoma. However, despite progress these therapies merely gave encouraging results. Several mechanisms of drug resistance have been identified using *in vivo* and *in vitro* studies (Table 1).[13,17,30-32,34,35,50,5154,57-60,62,63-72,78-90] In light of the evidence gained till date, it appears that the lethality of malignant melanoma is mainly due to the ability of these tumor cells to resist chemotherapy. Further a deeper understanding of the chemical nature of the resistance could improve the prospects of melanoma therapy. Such studies might lead to a novel approach for overcoming chemoresistance in melanoma by modulation of apoptotic pathways. Further *in vitro*, *in vivo* and clinical controlled studies are required to establish this hypothesis.

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