DNA repair pathways in drug resistance in melanoma

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Metastatic melanoma has a poor prognosis due to resistance to multiple chemotherapy regimens. The mainstay of treatment remains dacarbazine, with cisplatin being a commonly used alternative. Melanoma displays marked resistance to the DNA-damaging effects of these drugs. Intrinsic and acquired resistance involves multiple cellular pathways of damage recognition, repair and apoptosis. Increased understanding of these pathways is identifying novel targets that it is hoped will make inroads into the treatment of this lethal disease. ***Anti-Cancer Drugs*** **15:421–426 © 2004 Lippincott Williams & Wilkins.**

Anti-Cancer Drugs 2004, **15**:421–426

Keywords: DNA repair pathways, drug resistance, melanoma

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Received 22 December 2003 **Revised form accepted** 20 January 2004

Introduction

Melanoma is curable in 85% of cases by surgical resection. However, metastatic disease remains incurable, has a short median survival and is characterized by resistance to multiple cytotoxic agents [1,2]. The alkylating agent dacarbazine (DTIC) is the mainstay of treatment, but benefits are modest, with responses occurring in only 15% of cases and lasting a median of 6 months [3]. The oral agent temozolomide (TMZ) shares the same active moiety as DTIC and with its favorable side-effect profile is commonly used in the treatment of metastatic melanoma. It does not, however, have greater efficacy than DTIC in phase III trials [4].

Other cytotoxic drugs with activity in melanoma include the nitrosoureas [5], platinum analogs [6,7], vinca alkaloids [8] and taxanes [9]. The response rates from these treatments are no better than those achieved with the methylating agents and attempts to improve survival with combination chemotherapy have been unsuccessful [3,10]. Furthermore the combination of chemotherapy and immunotherapy has not led to an increase in overall survival in phase III trials, despite promising phase II results [11–13].

The poor response to chemotherapy and the observation that the incidence of melanoma continues to increase at a greater rate than for any other malignancy illustrate why new therapies are desperately needed [14].

The intrinsic and acquired resistance of melanoma is an area of intense investigation. This review will concentrate on how DNA repair mechanisms in melanoma contribute to resistance to the most commonly used cytotoxics and how greater understanding of resistance to these drugs is leading to novel therapeutic strategies.

From DNA damage to cell death

Cytotoxic-induced cell death secondary to DNA damage results from the activation of multiple pathways, starting with recognition of DNA damage and culminating in programmed cell death. Failure at any point along this pathway can lead to resistance. Increased or faulty DNA repair mechanisms and failure of apoptosis are significant problems to the efficacy of DTIC, TMZ and cisplatin (Fig. 1).

DNA repair pathways

O^6-methylguanine-DNA methyltransferase (MGMT)

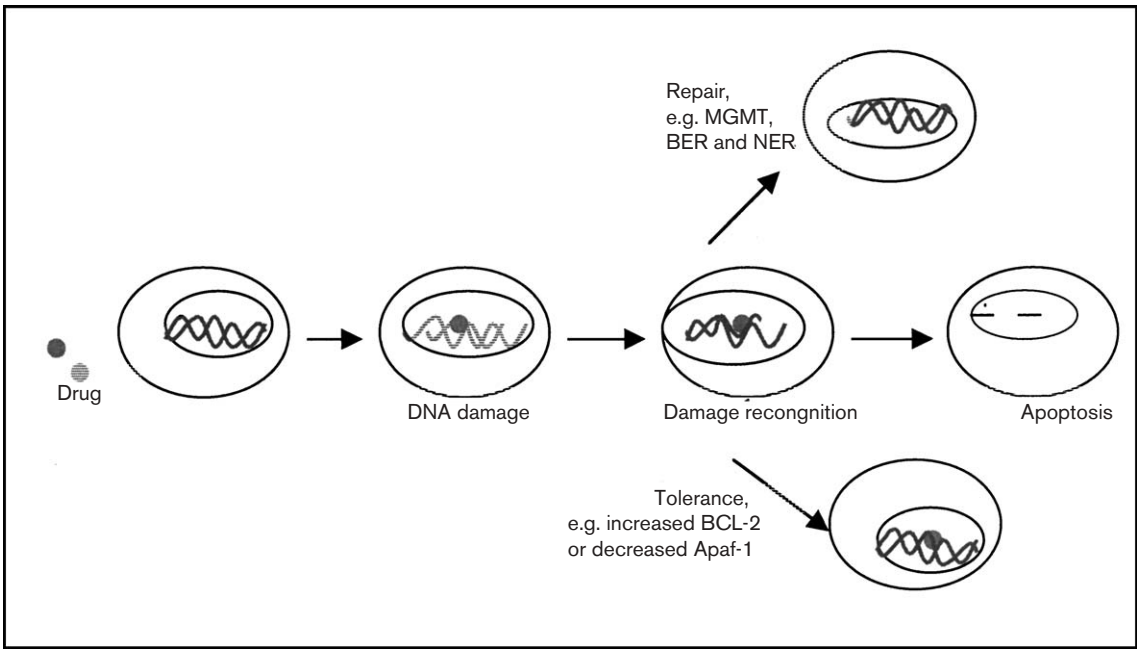
The active moiety in both DTIC and TMZ is 5-(3-methyltriazen-1-yl)imidazole-4-carboximide (MTIC), which methylates DNA bases via a methyldiazonium ion [15]. Damage occurs at multiple sites, but the most significant is methylation at the O^6 and N^7 positions on guanine [16]. In the former instance DNA replication leads to the formation of O^6-methyl G:T mismatches, which are recognized but perpetuated by the mismatch repair pathway, culminating in the engagement of apoptosis [17].

MGMT is a major cause of resistance to methylating agents. MGMT is a ubiquitously expressed DNA repair protein that is vital in the maintenance of the integrity of DNA. Unfortunately it is this protective effect that can also lead to resistance to the therapeutic effects of methylating agents, as damage induced by these drugs provides a perfect substrate for MGMT [18].

MGMT is conserved through evolution and has unique features that set it apart from other DNA repair mechanisms. MGMT is the only mechanism by which O^6-alkylguanine lesions are repaired with no redundancy in this DNA repair process [18]. Unlike other DNA repair

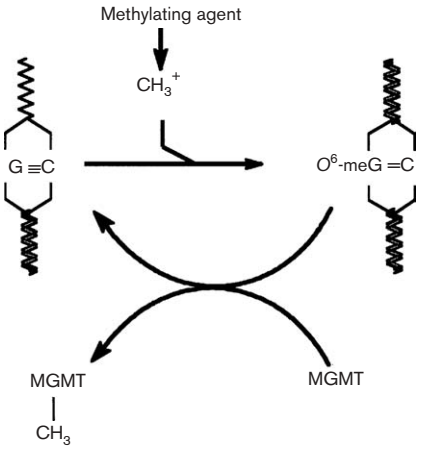
0959-4973 © 2004 Lippincott Williams & Wilkins DOI: 10.1097/01.cad.0000127665.74096.93

Fig. 1



Schematic diagram of the effects of chemotherapy and important resistance mechanisms in melanoma. Following entry into the cell, the effects of DNA-damaging drugs are recognized and DNA repair initiated. Cell death, where this occurs, is usually by apoptosis. Resistance can occur due to repair of the damage, failure to engage apoptosis or failure to recognize damage. In the latter cases, the cell can survive with new mutations.

Fig. 2



Mode of action of MGMT.

mechanisms, MGMT does not activate a pathway, but instead recognizes and repairs adducts at the O^6 position of the guanine in a suicidal stoichiometric reaction (Fig. 2). The inactivated protein is then ubiquitinated and degraded by proteosomes [19].

MGMT is expressed in the nucleus and cytoplasm of all cells, but the expression varies amongst different tissues. MGMT expression in tumors loosely correlates with the tissue of origin, but has commonly been shown to be higher [20]. Cancers that express high levels of MGMT include breast, stomach and lung cancer, with melanoma and gliomas having relatively low levels of MGMT [20].

In preclinical studies, expression of MGMT in tissue culture cell lines and in xenografts from tissue culture cell lines correlates with resistance to O^6 alkylating agents [21,22]. MGMT-induced resistance can be overcome by MGMT inhibition with a pseudosubstrate such as O^6-benzylguanine (O^6-BG) or following MGMT depletion after exposure to a methylating agent such as streptozotocin [21,23–25].

In the clinical setting the expression of MGMT has been shown to be inversely correlated with improved response to BCNU and overall survival in patients with gliomas, but the relationship is less clear in patients with melanoma [26]. One study has found an association between the pretreatment expression of MGMT and subsequent response to DTIC, but this was not confirmed by a study in patients treated with TMZ [27,28]. This apparent anomaly may be due to failure of downstream pathways such as engagement of apoptosis. The contribution of MGMT to melanoma resistance to methylating agents will be defined better with the results from clinical trials of inhibitors of MGMT that are now under way.

Attempts to overcome repair of O^6-methylguanine adducts have centered on depletion and inhibition of MGMT. MGMT is depleted after exposure to alkylating agents, as the protein is consumed in repairing DNA damage. This reduced expression of MGMT has been exploited in trials using combination treatment aimed at first reducing MGMT expression and then exposing the tumor to a further alkylating agent timed to coincide with the MGMT nadir. Whilst preclinical studies using carmustin-resistant cells demonstrated an increased sensitivity to carmustine if pretreated with streptozotocin, in a phase II study of this drug combination there was a disappointing response rate of 13% [29]. A trial using DTIC followed by fotemustine to treat patients with stage 4 melanoma showed improved response rates in groups receiving higher doses of DTIC. Greater MGMT depletion was evident at the time of fotemustine administration with higher doses of DTIC, appearing to vindicate the approach. However, this was achieved at the expense of increased myelosuppression and did not translate into improved survival [30].

Giving TMZ every 4 h, rather than every 24 h, might be expected to lead to enhanced activity, since this corresponds to the MGMT nadir following exposure to DTIC and TMZ [31,32]. In a phase II trial of 30 patients, TMZ every 4 h increased DNA methylation in peripheral blood mononuclear cells compared with conventional daily dosing. MGMT was also depleted more, but again significant myelosuppresion was found and overall survival at a median 6 months proved disappointing [33].

Direct inhibition of MGMT using pseudosubstrates is now being evaluated clinically. These agents are not in themselves myelotoxic, leading to the hope that the limiting toxicity experienced with the approaches above will be avoided. In a phase I trial using O^6-BG with carmustine, the dose-limiting toxicity was again myelosuppression and the carmustine dose could not be escalated above 25% of that normally used [34]. An alternative molecule O^6-(4-bromo)thenylguanine (Patrin-2) has been used in conjunction with TMZ. The maximum tolerated dose of TMZ was 150 mg/day, 75% of the single-agent dose. This combination of Patrin-2 and TMZ is currently being evaluated in melanoma and colorectal cancer. These studies will determine whether pseudosubstrate inhibitors offer an increase in therapeutic index for O^6-alkylating agents.

Base excision repair (BER)

The BER pathway also repairs lesions induced by methylating agents [35,36]. N^7-methylguanine adducts, which are formed at a greater frequency than O^6-guanine lesions are rapidly repaired by BER such that, despite their potential cytotoxicty, their therapeutic significance is limited. Inhibition of BER might, therefore, enhance the clinical efficacy of methylating agents.

A potential target for inhibition in the BER pathway is poly(ADP-ribose) polymerase (PARP). PARP is a nuclear enzyme that has a dual role in the repair of DNA damage and recruitment of apoptosis. Activated by DNA strand breaks, PARP recruits components of the BER complex to the damaged area. PARP has an integral role in the repair of DNA damage, although not crucial for cell survival in the absence of DNA damage [37].

PARP inhibitors have been in development for nearly two decades. Preclinical studies have demonstrated the potentiation of methylating agents in combination with PARP inhibitors, but in the clinical setting the lack of sensitivity and potency has limited their efficacy [38]. PARP is an abundant enzyme and without total inhibition potentiation of cytotoxic effects is limited. However, the development of more potent inhibitors used in conjunction with cytotoxic drugs in melanoma may improve the clinical efficacy of methylating agents. Trials with new potent inhibitors in conjunction with methylating agents are underway [39].

Nucleotide excision repair (NER)

Larger DNA distorting damage, e.g. as induced by cisplatin, is repaired by the NER pathway. The general steps of damage recognition, recruitment of the repair apparatus, excision of the damage and gap repair are employed [40]. In melanoma, the efficacy of cisplatin is limited [7]. This is in stark contrast to the efficacy of cisplatin in germ cell tumors, with cure rates of over 90% [41]. Analysis of NER in germ cell tumors reveals similarities with cells derived from the DNA repair disorder xeroderma pigmentosum. This disease is characterized by UV sensitivity and high incidence of skin cancers, with reduced expression of xeroderma pigmentosum A protein, a component of the NER pathway [42,43]. In ovarian cancer, expression of another NER protein, ERCC-1, correlates with cisplatin sensitivity [44]. The association between components of the NER pathway and melanoma resistance to chemotherapy has yet to be established.

Mismatch repair (MMR)

Cisplatin adducts are recognized by MMR complexes and resistance to the drug is associated with loss of expression of hMLH1, hMSH2 or hMSH6. Furthermore, re-expression of hMLH1, e.g. by reversal of promoter hypermethylation using 5-azacytidine, results in increased sensitivity to cisplatin. Single base errors and short nucleotide repeats are recognized and repaired by MMR. Germline loss of this repair pathway is associated with hereditary non-polyposis coli, with defects in the MMR also being recognized in sporadic colon and endometrial tumors. The loss of the MMR has been linked to acquired tumor resistance, e.g. in ovarian cancer, due to failure of the cell to appropriately enter apoptosis in the presence of DNA damage [45].

In melanoma, loss of MMR is not commonly described. Previous studies have not found MMR gene mutations and microsatellite instability is infrequent [46]. One series examining primary melanoma specimens has described loss of MMR expression with increasing Clarke's level [47]. Loss of MMR repair has been proposed as a mechanism for acquired resistance in some patients. Studies of melanoma cell lines with acquired chemoresistance show loss of MMR proteins in 30–70% of cases, but a clinical corollary has yet to be demonstrated [48].

Double-strand break (DSB) repair

Double-strand DNA defects can occur endogenously or via exposure to exogenous DNA-damaging agents and are amongst the most fatal DNA lesions. Unrepaired, DSBs can result in nucleotide loss and chromosome rearrangements, which commonly occur in cancer [49]. DSBs are repaired by two main mechanisms: homologous recombination and non-homologous end joining repair. In melanoma loss of Ku70 and Ku80 (a component of the latter pathway) have been associated with early melanoma progression [50]. Cisplatin sensitivity has been associated with altered Ku binding, but again clinical data in melanoma are lacking [51]. The role of homologous recombination in determining platinum sensitivity in the clinic is difficult to measure, as to date functional assays from tumor cell cultures are required.

Apoptosis

Engagement of programmed cell death is the final common pathway for many chemotherapy effects, without which the damage induced by cytotoxic chemotherapy will be unavailing. Metastatic melanoma has the hallmark of defective apoptosis with marked resistance to chemotherapy. Intrinsic and acquired damage to the multiple pathways involved in cell survival and apoptosis are being identified in melanoma (e.g. activating mutations of the *ras* oncogene, loss of PTEN regulatory control of Akt, up-regulation of Bcl-2 and epigenetic silencing of Apaf-1). These defects may account for the wide-ranging resistance displayed, but are also emerging as new targets for treatment. Therapeutic approaches designed to favor the path towards apoptosis include antisense oligonucleotide directed against Bcl-2, inhibition of the transcription factor ATF-2, the use of decitabine to reverse epigenetic silencing and proteasome inhibitors.

In melanoma, the expression of Bcl-2 inversely correlated with response to treatment and survival in some studies, although down-regulation in melanoma progression has also been reported [52]. The development of an effective antisense oligonucleotide to Bcl-2 mRNA has meant that its role can be evaluated in the clinic. In a phase I–II trial combining the Bcl-2 antisense oligonucleotide with dacarbazine, responses occurred in six of 14 patients with estimated median survival of greater than 12 months. This was coupled with evidence of a decrease in the tumor expression of Bcl-2 and increase in apoptosis [53]. Results from a large phase III study have recently been reported, comparing DTIC chemotherapy alone or with oblimersen (as the oligonucleotide is known). Relapse-free and overall survival favored the combination arm, although only statistically significant in the former case (H. Pehamberger, pers. commun.). A full trial report is awaited. Targeting ATF-2 has potential for the future as it has been associated with melanoma proliferation and resistance to radiotherapy. Bhoumik *et al.* have demonstrated inactivation of ATF-2 via an ATF-2 peptide altered the ATF-2/Jun balance, sensitizing melanoma cells to treatment [54].

Less-specific measures with multiple potential targets includes use of proteasome inhibitors or reversal of epigenetic silencing. Proteasome inhibition leads to an as yet poorly defined initiation of apoptosis, with increases in caspases and inactivation of NK-κB. Preclinical studies of the proteasome inhibitors have shown some promise, although much of the melanoma work to date has been *in vitro* [55,56].

In melanoma, loss of Apaf-1 expression has been demonstrated through allelic loss or epigenetic silencing [57]. The loss of Apaf-1, a key component in the apoptotic pathway, has the capacity to engender resistance to chemotherapy. Epigenetic silencing also affects other DNA repair and apoptotic protein expressions (e.g. DAPK). Decitabine, a cytotoxic discarded on the basis of toxicity in the 1980s, inhibits and can reverse DNA methylation at tolerable doses. Recently there has been renewed interest in decitabine as a means for reversing epigenetic silencing [58]. A number of clinical trials are in progress, with results potentially applicable to melanoma.

Conclusion

This brief review has concentrated on the main DNA repair mechanisms that contribute to the resistance of metastatic melanoma and on potential ways to overcome them. Improved understanding of these mechanisms is leading to the identification of novel therapeutic targets. The multiple intrinsic and acquired defects that occur in metastatic melanoma illustrates the considerable challenges ahead, but there are perhaps grounds for hoping that we may soon be able to make headway.

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