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Involvement of ABC Transporters in Melanogenesis and the Development of Multidrug Resistance of Melanoma

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Abstract

Because melanomas are intrinsically resistant to conventional radiotherapy and chemotherapy, many alternative treatment approaches have been developed such as biochemotherapy and immunotherapy. The most common cause of multidrug resistance (MDR) in human cancers is the expression and function of one or more ATP-binding cassette (ABC) transporters that efflux anticancer drugs from cells. Melanoma cells express a group of ABC transporters (such as ABCA9, ABCB1, ABCB5, ABCB8, ABCC1, ABCC2, and ABCD1) that may be associated with the resistance of melanoma cells to a broad range of anticancer drugs and/or of melanocytes to toxic melanin intermediates and metabolites. In this review, we propose a model (termed the ABC-M model) in which the intrinsic MDR of melanoma cells is at least in part due to the transporter systems that may also play a critical role in reducing the cytotoxicity of the melanogenic pathway in melanocytes. The ABC-M model suggests molecular strategies to reverse MDR function in the context of the melanogenic pathway, which could open therapeutic avenues towards the ultimate goal of circumventing clinical MDR in patients with melanoma.

Keywords

Melanoma; multidrug resistance; melanosome; ATP-binding cassette; transporters; ABCB5; ABCC2

Introduction

Melanomas, which rank 6th in incidence of all cancers, are usually fatal after tumor cells metastasize. They are responsible for more than 8,000 deaths a year in the United States (www.cancer.gov). The management of advanced stages of melanoma is quite difficult because the cells are usually resistant to conventional therapies such radiation therapy and chemotherapy. For these reasons, many alternative therapies have been proposed or developed based on mutations, signaling pathways, and the immunological and stem cell properties of melanoma cells in attempts to improve therapeutic outcomes (Fang et al., 2005; Lewis et al., 2006; Rosenberg and Dudley, 2009; Schatton et al., 2008; Tsao et al., 2004; Zabierowski and Herlyn, 2008). There are some promising studies using adoptive cell therapy in patients with metastatic melanomas (Rosenberg and Dudley, 2009). Nonetheless,

therapeutic resistance is still a major obstacle to the therapy of patients with metastatic melanomas.

Deciphering the mechanisms underlying the resistance of melanomas to chemotherapy could significantly improve the outcomes of current therapies and should suggest novel approaches to new therapies. The precise causes that underlie the therapeutic resistance of melanomas are not well understood, and they are likely to be mediated by diverse mechanisms. Those mechanisms include increased DNA repair in response to DNA-damaging agents, over-expressed anti-apoptotic proteins such as Bcl-2, altered expression of oncogenes such as RAS and BRAF or the tumor suppressor p53, methylation-mediated silencing of the APAF-1 gene, (Chin et al., 2006; Helmbach et al., 2002; Soengas and Lowe, 2003), and increased levels of endogenous nitric oxide (Tang and Grimm, 2004).

Although understanding these general resistance mechanisms is important, that does not explain why melanomas are particularly insensitive to chemotherapeutic regimens compared to a broad spectrum of nonmelanoma cancers. One intuitive explanation for this difference is that melanomas may be armed with additional resistance mechanisms related to their derivation from pigmented melanocytes compared with nonmelanoma cancers. A major difference between melanoma and nonmelanoma cancer cells lies in a unique subcellular organelle termed the melanosome, a lysosome-related organelle modified for melanin synthesis (Hearing, 2005; Raposo et al., 2002; Valencia et al., 2006), which has been implicated in drug sequestration and export (Chen et al., 2006; Chen et al., 2009).

The goal of this review is to unravel the cellular and molecular mechanisms underlying drug sensitivity and resistance in melanoma cells. We elucidate the role of melanosome dynamics (including the biogenesis of melanosomes, melanosome status, and the integrity of melanosome structures) in determining the drug sensitivity of melanoma cells. We propose a melanogenic model that relates drug sensitivity to melanogenesis based on existing cellular and genetic evidence. We further analyze the potential roles of ATP-binding cassette (ABC) transporters in melanogenesis, in detoxifying endogenous melanogenic cytotoxicity (EMC), and in conferring drug resistance. An integrative model (termed the ABC-M model) that considers the role of ABC transporters during melanogenesis is introduced to account for intrinsic and acquired multidrug resistance (MDR) mechanisms in melanoma cells. Finally, rational molecular interventions based on the ABC-M model are proposed.

Melanosome biogenesis, melanin synthesis, and EMC

Melanogenesis includes three major components: melanosome biogenesis, melanin synthesis, and EMC-related homeostasis. Melanosome biogenesis can be classified into four distinct stages of maturation (I, II, III, and IV) based on melanosomal morphologies (Hearing, 2005; Raposo et al., 2002). Stage I and II melanosomes are termed premelanosomes since melanin synthesis has not yet begun. Stage I melanosomes are closely related to lysosomes because they share a lysosomal lineage (Hearing, 2005; Orlow, 1995; Raposo et al., 2002; Raposo et al., 2001; Valencia et al., 2006). Typical stage II melanosomes have a morphology similar to that of an American football, containing elongated and highly organized fibrillar matrices used as substrates for melanin accumulation following its synthesis. Active melanin synthesis occurs in stage III melanosomes, and results in the deposition of black electron-dense pigment on the fibrillar matrix. When the internal matrix of a melanosome is completely filled with melanin, it no longer has discernible internal filaments at the electron microscope (EM) level, and the melanosome reaches a mature stage (i.e., stage IV).

More detailed staging of melanosomes can be obtained from EM images when melanosomes undergo inter-stage transitions from stage I to IV. These additional stages are I-II, II-III,

and III–IV (Chen et al., 2009; Chen et al., 2006). Moreover, late-stage melanosomes (stages III and IV) are normally transferred to keratinocytes in normal skin. However, that process may be impaired in melanoma cells. We frequently observed that stage IV melanosomes have damaged membranes and leak melanin into the cytoplasm, a morphological indicator of EMC (Chen et al., 2009). Here, we designate this type of mature melanosome with damaged membrane structures or with severe EMC as stage IV_d melanosomes. Elucidation of melanosomal classification could enable us to clarify the roles of melanosomes in producing or detoxifying EMC during melanin synthesis, which is potentially an extremely toxic process.

During melanogenesis, melanin intermediates and byproducts that are highly toxic to cells are generated. Those intermediates are usually small molecules, such as 5,6-dihydroxyindole (DHI), 5,6-dihydroxyindole-2-carboxylic acid (DHICA), quinones, indole-quinones and hydrogen peroxide (Hearing, 2005). The escape of those toxic substances from melanosomes into the cytoplasm, nucleus, and mitochondria induces cytotoxic effects that are deleterious to melanoma cells (Chen et al., 2009). We speculate that one of the roles of melanosomes in normal melanocytes is to provide an environment where these chemical reactions can occur and to permit the formation of melanin biopolymers, which in turn inactivate the process and keep the cytotoxic effects under control. Hence, detoxification of EMC appears to be a double-edged sword. In the case of melanomas, the detoxifying functions of melanosomes might render cells resistant to anticancer drugs.

It has been shown that the melanogenic system is involved in the regulation of drug sensitivity. We previously demonstrated that melanosomes are involved in drug trapping and export (Chen et al., 2006). Recently, we studied the influence of melanosome dynamics in the regulation of drug sensitivity in the melanosome biogenesis pathway (Chen et al., 2009). Moreover, tyrosinase-related protein-2 (TYRP2), a melanogenic enzyme, was shown to confer resistance to CDDP in melanoma cells (Chu et al., 2000; Pak et al., 2001; Pak et al., 2000). Thus, the sum of these studies suggests that the melanogenic system is likely a major regulator of drug sensitivity, which implies that therapeutic approaches directed at this system may pave the way to circumventing drug resistance in melanomas.

Melanogenic model of drug sensitivity

Based on our research, we propose a mechanistic model that relates drug resistance to the melanogenic pathway (Figure 1). This model depicts an intrinsic linkage between melanogenesis and drug sensitivity. Based on the sequence of melanosome biogenesis described above, we might consider melanogenesis to have three distinct phases (i.e., I to III) according to differences in drug sensitivity (Chen et al., 2009). Briefly, phase I includes biogenesis of stage I and II melanosomes. Phase II covers melanosome stages II–III, III, and III–IV, whose stability is important for the maintenance of homeostasis in melanocytic cells. Finally, phase III involves mainly stage IV and IV_d melanosomes. The dynamic nature of the melanogenic pathway is thought to underlie the differential drug sensitivity in these distinct phases (Chen et al., 2009).

To explain in more detail, Phase I is the decisive phase that determines the fate of melanosomes. As the biology of stage I melanosomes reflects some of the functions of lysosomes, they are considered to be lysosome-related organelles. Interestingly, lysosomes have been implicated in the resistance to CDDP of human ovarian carcinoma cells through altered lysosomal trafficking, exosomal CDDP trapping, and enhanced export of exosomes (Safaei et al., 2005a; Safaei et al., 2005b). Likewise, in phase I, melanosomal protein trafficking and sorting of Pmel17 is essential to the generation of the melanosomal matrix essential to the formation of stage II melanosomes (Valencia et al., 2006). No doubt,

melanosomes at this stage possess the ability to trap and export cytotoxic drugs such as CDDP. However, an increase in numbers of stage II and II–III melanosomes would augment their capacity to confer drug resistance. In contrast, a deficiency in the biogenesis of stage II and II–III melanosomes would make cells more sensitive to cytotoxic drugs. Existing genetic evidence supports the above statements. For example, Wei and colleagues have recently shown that several mutations which affect melanosomal components (e.g., gp100/Pmel17) required for the formation of stage II melanosomes enhance sensitivity to anticancer drugs such as etoposide and CDDP (Xie et al., 2009). Therefore, early stage melanosomes serve as a threshold reference concerning drug sensitivity. Thus, the directionality of melanosome biogenesis determines melanosome dynamics, which in turn influences the drug sensitivity of melanoma cells (Figure 1).

Phase II represents a transition stage between early-stage (stage I and II) and late-stage (stage IV) melanosomes. In phase II, melanosomes are predominantly at stage III, and are likely to be involved in drug resistance since they possess a greater capacity to trap cytotoxic drugs in the nascent melanin (Chen et al., 2009; Chen et al., 2006). Consistently, drug-resistant cells treated with a sublethal dose of CDDP (6.7 μ M) showed the presence of melanosomes predominantly at stages II–III and III (Chen et al., 2009). The pharmacological modulation of melanosomes by 1-phenyl-2-thiourea (PTU), a tyrosinase inhibitor that reduces melanin formation, to stage II or II–III significantly increases resistance to CDDP by 12-fold (Chen et al., 2009). These studies are consistent with the increased drug resistance that accompanies increased melanogenesis, particularly with increased melanosome biogenesis, at the middle phase of our model.

At phase III, the melanosome maturation process likely generates maximal EMC. The degree of EMC is associated with the integrity of melanosomes and the ability of the cells to execute an autophagy program on damaged melanosomes. At this phase, melanoma cells should be more susceptible to cytotoxic drugs. For example, melan-p1 melanocytes are deficient in *p* gene transcripts (which encode the pink-eyed dilution protein) that result in dramatic hypopigmentation. Orlow and co-workers stably transfected the *p* gene into the cells to give rise to highly pigmented melan-p1⁺P cells due to the rescue of tyrosinase-containing melanosomes (Chen et al., 2002). Concomitantly, these highly pigmented cells were more sensitive both to arsenic and to CDDP compared with control untransfected melan-p1 melanocytes (Staleva et al., 2002). In addition, transfection of TYRP2 cDNA confers resistance to CDDP in melanoma cells (Chu et al., 2000) and abrogates UV(B)-induced apoptosis (Nishioka et al., 1999). The underlying resistance mechanisms may involve the ability of TYRP2, in cooperation with TYRP1, to repress the EMC induced by over-expression of tyrosinase (Rad et al., 2004).

General therapeutic strategies based on the melanogenic model

Based on the model, there are several key components that are involved in the melanogenic pathway that could be sequentially utilized for the therapy of melanoma (Figure 1). Initial strategies could prevent or inhibit melanosome-mediated cytotoxic trapping and export. This may be of particular interest for targeting melanoma cells containing abundant II and II–III melanosomes (Chen et al., 2006). Furthermore, manipulating or enhancing the EMC could be exploited for therapy at phase III. Although the melanogenic model provides such general strategies, it lacks specific targets for molecular intervention. Multiple ABC transporters whose expression has been associated with MDR are expressed in melanoma cells, which might represent ideal molecular targets for therapeutic purposes.

ABC transporters and MDR in melanoma

ABC transporters belong to a superfamily of integral membrane proteins and convey structurally diverse molecules across biological membranes in an ATP-dependent manner. The substrates of the transporters range from small ions, sugars, and peptides to more complex organic molecules (Dean and Annilo, 2005; Deeley et al., 2006; Doyle and Ross, 2003; Gottesman et al., 2002; Gottesman and Ling, 2006; Robey et al., 2007; Szakacs et al., 2006). Forty-eight ABC transporter proteins have been identified so far in the human genome, of which ABCB1, ABCC1, and ABCG2 have been well characterized in terms of their capacities to confer the MDR phenotype in vitro and in vivo, and their potential roles in mediating clinical MDR in a broad range of human cancers.

A cluster of ABC transporters is expressed in melanomas, including ABCA9, ABCB1, ABCB5, ABCB8, ABCC2, and ABCD1 (Chen et al., 2005; Elliott and Al-Hajj, 2009; Frank et al., 2003; Szakacs et al., 2004). However, the functions of the majority of those transporters in melanoma cells have not been clarified. Thus, elucidation of their roles in melanoma drug resistance and in melanogenesis could allow us to design more specific and efficient inhibitors for molecular intervention of MDR in melanomas.

ABCB1 expression and MDR in melanomas

ABCB1 (P-glycoprotein; P-gp) is the best-characterized ABC transporter in many types of cancers. However, endogenous *ABCB1* (*MDR1*) mRNA levels were found to be low or undetectable in melanomas (Goldstein et al., 1989). Only one (SK-MEL-28) of the five melanoma cell lines (HT-144, SK-MEL-28, SK-MEL-5, Me665/2/21, and Me665/2/60) tested by Miracco and colleagues (Miracco et al., 2003) and, using real-time RT-PCR, only two (M14 and SK-MEL-5) of the eight melanoma cell lines (M14, LOX-IMVI, MALME-3M, SK-MEL-2, SK-MEL-5, SK-MEL-28, UACC-62, and UACC-257) in the NCI-60 cell panel expressed *ABCB1* mRNA (Szakacs et al., 2004). These differences in reported results indicate that further verification is necessary. Consistently, the monoclonal antibody C219 that recognizes P-gp had 3% and 4% positivity in 37 primary melanomas and in 27 melanoma metastases, respectively (Fuchs et al., 1991). In contrast to those findings, P-gp expression has been frequently reported in non-cutaneous melanomas such as ocular (42%) and uveal tract (80%) melanomas (Dunne et al., 1998; McNamara et al., 1996). The ability of P-gp to confer MDR in melanoma cells was confirmed by transfection of *ABCB1* (*MDR1*) cDNA into melanoma cells (Lincke et al., 1990). Nevertheless, the sum of those studies suggests that P-gp is not responsible for the intrinsic drug-resistant phenotype in cutaneous melanomas, but may be an important prognostic factor in some specific types of melanomas.

It is generally believed that P-gp functions as an ATP-dependent efflux pump in the plasma membrane. However, P-gp might also function in membranes of subcellular organelles to exert its function, and supporting that, Molinari et al. reported that mislocalized P-gp sequesters P-gp substrates into subcellular organelles (Molinari et al., 1998). However, the frequency of this mechanism and its role in melanosomes or lysosomes could not be validated due to the small number of samples in that study. Furthermore, no elevation of P-gp expression or function has been reported for melanoma patients before or after chemotherapy. Thus, the role of P-gp in the MDR of melanomas remains to be established, and other ABCB subfamily members may be involved.

ABCB5 expression in melanocytes and in melanomas

The most frequent ABC mRNA transcripts previously isolated from melanoma cDNA libraries were the *ABCB5 α* and *ABCB5 β* isoforms (Chen et al., 2005), neither of which

encodes the full-length ABCB5 protein. This full-length ABCB5 protein is highly homologous to ABCB1. *ABCB5 α* mRNA predicts a 15-KD protein that lacks an intact domain for a half-transporter protein (Chen et al., 2005), whereas *ABCB5 β* potentially encodes a more-than-half transporter protein. The protein sequences of those two ABCB5 isoforms have not been determined due to the lack of specific antibodies that could be used to immunoprecipitate ABCB5 (Chen KG and Gottesman MM, unpublished data). The *ABCB5 α/β* isoforms are predominantly expressed in melanocytes, retinal pigment epithelial (RPE) cells, and melanomas (Chen et al., 2005). It has also been shown that *ABCB5 α* mRNA is expressed both in the testes and in the uterus (Langmann et al., 2003). However, *ABCB5 α/β* mRNAs were undetectable in two amelanotic melanomas (M14 and LOX-IMVI) that are deficient in melanin synthesis (Chen et al., 2005; Szakacs et al., 2004), which suggests that the two *ABCB5* mRNA isoforms are associated with melanogenesis. Another proposed physiological role of *ABCB5 β* is to regulate the fusion of progenitor cells (Frank et al., 2003). It is worth noting that not all melanotic melanomas express *ABCB5 β* and we have reported that highly pigmented MNT-1 melanoma cells do not express such transcripts (Chen and Gottesman, 2005). *ABCB5 α/β* mRNAs are regulated by many cytotoxic drugs, a few of which are known substrates for P-gp (Chen et al., 2005). Hence, *ABCB5 α/β* appear to have distinct patterns of drug responses compared with *ABCB1*. Typical multidrug-resistant cell lines (e.g., MES-SA/Dx5 and KB-V1), which were selected in a step-wise fashion with doxorubicin and vinblastine, respectively, did not express *ABCB5 α/β* (Chen et al., 2005). A possible function of ABCB5 α is suggested by using an siRNA that targets the 3' untranslated region of *ABCB5 α* mRNA (Huang et al., 2004). The subsequent down-regulation of *ABCB5 α* and possibly other transcripts including this 3' untranslated region resulted in a gain of drug sensitivity to camptothecin 10-OH, 5-fluorouracil, and mitoxantrone (Huang et al., 2004), suggesting, but not proving that *ABCB5 α* is involved in drug resistance. *ABCB5 β* was suggested to confer drug resistance to doxorubicin (Frank et al., 2005). That conclusion was based on ABCB5⁺ cells isolated by a monoclonal antibody against ABCB5, but no transfection data were generated in that study. We have recently stably transfected *ABCB5 β* into KB-3-1 cervical adenocarcinoma cells and into UACC-257 melanoma cells. Our preliminary data indicate that *ABCB5 β* alone is unable to confer drug resistance to these cells (Gillet et al., 2009). Thus, the role of both *ABCB5 α* and *ABCB5 β* in conferring MDR to melanoma cells remains to be established. Nonetheless, expression of a full-length ABCB5 cDNA is essential to understand its function at the protein level.

The locus of the putative full-length *ABCB5* gene is at chromosome 7p15. We identified the full-length *ABCB5* cDNA sequence at that locus 5 years ago using a bioinformatics approach (Chen KG and Gottesman MM, unpublished sequence). We subsequently constructed the full-length *ABCB5* cDNA based on the above sequence information (Chen KG, Gillet JP, and Gottesman MM, unpublished data). The sequence identity was confirmed by a full-length *ABCB5* cDNA clone (NCBI accession number: AB353947) from the laboratory of Yoshikazu Sugimoto. The full-length *ABCB5* contains the mRNA sequence that potentially encodes a full-length ABCB5 transporter, which is similar to its homolog ABCB1 (P-gp). The deduced full-length ABCB5 has twelve transmembrane domains (TMDs), two nucleotide-binding domains (NDBs), and two ABC motifs (Figure 2). Northern blotting demonstrated that the *ABCB5* locus expresses multiple alternatively spliced transcripts ranging from 1.2- to 9.5-Kb, which includes a the 4.5-kb fragment, presumably the full-length *ABCB5* (Chen et al., 2005). We have recently expressed the full-length *ABCB5* in KB-3-1 and in UACC-257 cells. Our preliminary results indicate that the full-length *ABCB5*, in contrast to *ABCB5 β* , is able to confer drug resistance to multiple anticancer drugs that include anthracycline, mitoxantrone, and epipodophyllotoxin (Gillet et al., 2009). These data suggest that the structural integrity of ABCB5 is required to exert its function as a multidrug transporter. Over-expression of the ABCB5 protein is likely to be a major mechanism for the MDR of melanoma cells.

ABCB5 and melanoma stem cells

Cancer stem cells or tumor-initiating cells represent a small or rare subset of cells from bulk tumor populations. These cells are believed to have high tumorigenicity, possess self-renewal capacity, and have the potential to differentiate into related adult tissues. The existence of cancer stem cells in leukemia appears to be confirmed. The restrictive expression of ABCB1 (P-gp) and other putative ABC transporters within a subset of CD34⁺/CD38[−] progenitor cells enables both ABCB1 and CD34 to be used as stem cell markers to isolate leukemia stem cells in AML (Raaijmakers et al., 2006). These ABCB1 and CD34 positive leukemia stem cells were associated with poor prognosis in cancer patients, likely by rendering them resistant to reversal agents of MDR (Raaijmakers et al., 2006). In the case of melanomas, cancer stem cell properties were found in metastatic melanoma specimens and in cell lines when the cells were cultured in embryonic stem cell-based media (Fang et al., 2005). However, the existence of melanoma stem cells seems to be controversial because of the observed high frequency (~25%) of tumor-initiating cells derived from melanoma patients, as reported recently (Quintana et al., 2008). Ongoing investigations are focusing on several major subsets of melanoma cells that express stem-cell markers such as CD20 and CD133, exhibit slow-cycling, and have high ABC transporter activities (Zabierowski and Herlyn, 2008). Hence, a putative monoclonal antibody that recognizes ABCB5 was used to isolate melanoma stem cells (Schatten et al., 2008), and those ABCB5⁺ subpopulation cells were shown to have high tumorigenicity in a mouse model compared with the ABCB5[−] control. However, ABCB5 is ubiquitously expressed in pigment-producing cells (e.g., in normal human melanocytes and in the RPE) and in melanomas (Chen et al., 2005). Further genetic experiments using gene deletion and gene silencing approaches are needed to clarify the potential role of ABCB5 as a melanoma stem cell marker and its therapeutic potential as a molecular target.

ABCC subfamily members

ABCC (MRP) subfamily members actively transport structurally diverse compounds that include glutathione, glucuronide, and sulfate conjugates. These subfamily members possess the ability to confer resistance to a broad range of anticancer drugs and their conjugated metabolites, including a list of non-P-gp substrates such as platinum compounds, nucleotide analogs, and arsenical oxyanions (Deeley et al., 2006). Thus, the role of ABCC subfamily members in mediating the multidrug-resistant phenotype in melanomas is of particular interest, since melanoma cells are intrinsically resistant to some anticancer drugs (e.g., platinum compounds) that are associated with many ABCC subfamily members. Moreover, some ABCC (MRP) substrates are related to melanin intermediates and glutathione metabolism in pigment-producing cells.

The role of ABCC1 in the MDR of melanomas is unclear due to limited reports in the literature. ABCC1 was shown to cooperate with glutathione S-transferase M1 to help melanoma cells escape the cytotoxicity of vincristine (Depeille et al., 2005). Mislocalization of ABCC1 in a Golgi-like cellular compartment, and not on the plasma membrane, was associated with the trapping of anthracycline in the cytoplasm (Molinari et al., 1998). However, the frequency of this intracellular localization was not reported. Interestingly, such acquired mislocalization of ABCC1 was also found in KB-3-1 cells, derived from a HeLa cervical adenocarcinoma, that were selected for CDDP resistance (Liang et al., 2003). Further analysis indicated that the intracellular localization of ABCC1 is associated with the trans-Golgi network (Liang et al., 2003). It seems that the subcellular localization of ABCC1 represents a surrogate marker in KB-3-1 CDDP-resistant cells, which is somewhat different in melanoma cells where the 'mislocalization' of ABCC1 in melanoma cells is intrinsic. The

'mislocalized' ABCC1 might facilitate subcellular sequestering of melanogenic metabolites as well as anticancer drugs to subcellular organelles.

Elevated expression of ABCC2 (MRP2) was shown to cause CDDP resistance by reducing nuclear DNA damage, decreasing cell cycle G2-arrest, and increasing reentry into the cell cycle in CDDP-resistant MeWo CIS 1 cells (Liedert et al., 2003). Down-regulation of ABCC2 by anti-MRP2 hammerhead ribozymes enhanced drug sensitivity in the ovarian carcinoma line A2780RCIS (Materna et al., 2005). These data suggest that both ABCC2 and ABCB5 may be important molecular targets for the reversal of intrinsic MDR in melanoma cells.

Integration of ABC transporters into the melanogenic model

To better understand the complex mechanisms of MDR in melanomas, we have integrated ABC transporters into the melanogenic model (Figure 2). That integrated model (referred to as the ABC-M model) depicts alterations of drug sensitivity with progression through the melanogenic pathway. According to the ABC-M model, the entire ABC transporter network and/or the melanogenic pathway is involved in the regulation of drug sensitivity. This model explains the intrinsic mechanisms of MDR in melanoma cells. In nonmelanoma cancer cells, subcellular compartments, such as vesicles, endosomes, and lysosomes, are involved in subcellular drug trapping or sequestration. Besides those mechanisms, melanoma cells utilize additional and specific subcellular organelles (melanosomes) for these purposes. Several ABC transporters, such as ABCB5 and ABCC2, are endogenously expressed at high levels in melanoma cells. The distribution and regional intensity of these transporters in diverse subcellular organelles would constitute a buffer system that prevents nuclear or mitochondrial import of cytotoxic drugs (Figure 2).

In the ABC transporter system, a general observation is that full-length ABC transporters (e.g., ABCB1, ABCC1, and ABCC7) usually are localized on the plasma membrane, whereas half-ABC transporters (e.g., ABCB2/TAP1, ABCB3/TAP3, and ABCD3) function as homodimers or heterodimers on subcellular membranes (<http://nutrigene.4t.com/humanabc.htm>). In pigment-producing cells, a teleological hypothesis is that some ABC transporters, especially ABCB5 isoforms, might function as heterodimers on lysosomal and/or melanosomal membranes to trap melanin metabolites in those organelles, thereby protecting the cells from EMC generated by late-stage melanosomes (Figure 2). The full-length ABCB5 likely functions on the plasma membrane similar to its homolog ABCB1 (P-gp). Ongoing efforts are aimed at developing immunohistochemical grade antibodies against various ABCB5 epitopes, which would confirm the above hypothesis. Nonetheless, the ABC-M model provides potential specific molecular targets in the melanogenic pathway for the therapy of melanoma.

Therapeutic strategies based on the ABC-M model

In principle, MDR should be considered as a genetic disorder, because it has complicated genetic traits conferred by multiple molecular systems. These systems, to a large extent, are coordinately regulated. With regard to melanoma, therapeutic principles depend on the inhibition of multidrug transporters and on molecular intervention in the melanogenic pathway. Strategies for modulating ABC transporters have been extensively reviewed. The general principles based on the melanogenic model have been detailed above and in our recent study (Chen et al., 2009). Deliberately disrupting or inhibiting one major component (e.g., ABCB5) in melanomas is necessary but might not be sufficient to improve the therapeutic resistance. Simultaneous intervention of multiple ABC transporters is a logical step to minimize the redundancy of alternative mechanisms. ABCB1, ABCC2, ABCBA9,

and ABCD1 would be potential molecular targets, but that would depend on their expression profiles in individual melanoma patients.

Strategically, the design of therapeutic regimens should consider the three phases of the melanogenic pathway separately, because those phases have distinct features that may lead to different therapeutic outcomes (Chen et al., 2009). Combined therapeutic regimens often include multiple anticancer drugs plus or minus MDR inhibitors (Sikic et al., 1997). Importantly, such regimens should interfere with the melanogenic pathway at the appropriate phase, and should ultimately result in synergistic effects to enhance drug sensitivity. In fact, many anticancer drugs modulate melanogenesis by appreciably increasing melanin synthesis, e.g. thiotepa (Horn et al., 1989), paclitaxel (Bicamumpaka and Page, 1998), cisplatin (Chen et al., 2006), vinblastine (Chen et al., 2006), etoposide (Chen et al., 2006), and 5-fluorouracil (Cui et al., 2007). Moreover, cyclosporine A, an MDR inhibitor, decreased the pigmentation of cultured melanocytes via the down-regulation of tyrosinase expression (Lee and Kang, 2003). Regardless of the influence of anticancer drugs on pigmentation, the effects of these drugs (except CDDP) on the melanosome status have not been evaluated. We believe that verification of the effects of anticancer drugs on melanosomes should allow us to avoid the antagonistic effects of stage II and III melanosomes.

The use of inhibitors for subcellular organelles that are related to the melanogenic system could be an efficient way to enhance therapeutic efficacy. One related example is the use of proton pump inhibitors to directly inhibit lysosomal vacuolar- H^+ -ATPase (Luciani et al., 2004). Such inhibition resulted in increased extracellular and lysosomal pH, decreased drug retention in the cytoplasm, and enhanced nuclear drug influx, all of which sensitized melanoma cells to the effects of CDDP, 5-fluorouracil, and vinblastine (Luciani et al., 2004). To gain further insight into the effects of membrane protein inhibitors on EMC, we recently used the cyclosporin analog PSC-833 (also known as valspodar) to modulate EMC in melanotic melanoma cells (Chen et al., 2009). We found that PSC-833 inhibits cell growth by inducing large amounts of vacuolar structures and autophagosomes that contain damaged melanosomes (Chen et al., 2009). The enhanced autophagy can be interpreted as a cellular response to the increased EMC resulting from damaged melanosomes. The autophagosome-like vacuoles also contain other damaged subcellular organelles, indicating that PSC-833-mediated autophagy involved its interference with membrane transporter systems that are not specific to melanosomes (Chen et al., 2009). Our data also suggests that the mechanism by which PSC-833 suppresses melanoma cell growth involves its interaction with ABC transporters, potentially the P-gp homolog ABCB5.

In practice, therapeutic insights derived from the ABC-M model could be incorporated into current regimens to boost therapeutic efficacy, because some agents that we examined are widely used in oncology practice (e.g., CDDP) or are now in clinical trials (e.g., PSC-833). Mainstream regimens to treat advanced melanomas usually consist of anticancer drugs combined with biological modifiers (Tsao et al., 2004). For example, the CDT-II regimen (cisplatin, dacarbazine, and tamoxifen plus both high-dose IL-2 and interferon alfa-2b) showed a 44% response rate in advanced stage melanomas compared with the 27% response observed with CDT alone (Rosenberg et al., 1999). Again, the impact of components (besides CDDP) in the regimen on the melanosome stage is unknown in these patients. Our recent results showed that CDDP preferentially eradicates cells with stage IV melanosomes (Chen et al., 2009), but might not be suitable for treating patients with amelanotic melanomas or melanomas with predominant early-stage melanosomes.

Conclusions

The intrinsic MDR resistance and sensitivity in melanomas and in pigment-producing cells involves multiple ABC transporters and melanosome biogenesis. The three distinct phases in the melanogenic pathway can be differentially targeted for therapy of melanomas. For example, molecular therapy of melanomas may be achieved by increasing the EMC in melanoma cells via synergistic effects of anticancer drugs with inhibitors of ABC transporters that confer MDR. Modifications of current promising regimens or development of new protocols based on insights developed from the ABC-M model should improve the therapeutic outcome for patients with advanced melanomas.

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Figure 1. Schematic of the melanogenic model and cellular drug resistance mechanisms (A) Melanosome stages are divided into stages I, I–II, II, II–III, III, III–IV, IV, and IV_d (i.e., damaged or disrupted stage IV melanosomes). (B) Cellular model based on the biogenesis of melanosomes delineating alterations of EMC and drug resistance or sensitivity in three major phases of the melanogenic pathway. Arrows indicate the directionality of the cellular progress (i.e., progression of melanogenesis, development of drug resistance/sensitivity, alterations of EMC) (Adapted from Chen et al. JNCI, in press.)

Figure 2. Integrative model combining the ABC transporter system with the melanogenic pathway including melanosome biogenesis

(A) Putative full-length ABCB5 proteins (~140 Kd). (B) Intrinsic and acquired MDR associated with the ABC transporter system in melanocytes and melanoma cells: In normal pigment-producing cells such as melanocytes, the ABC transporter system (e.g., ABCB5 and ABCC2) regulates the homeostasis of melanocytes by trapping cytotoxic melanin intermediates into subcellular organelles such as small vesicles, endosomes, lysosomes, and melanosomes. In melanocytes, these drug-containing organelles are normally then exported from the cells via different mechanisms including exocytosis and melanosome export. In melanoma cells, these cancerous cells utilize the same system to confer MDR to chemotherapeutic drugs. Abbreviations: ATG, translation start codon; I to IV, stage I to IV melanosomes; Ly, lysosomes; Nu, nucleus; Nuc, nucleolus; PhM, autophagosome; TGA, translation stop codon; TM, transmembrane domain; WA, Walker A motif; WB, Walker B motif; WC, Walker C motif