

■ CELL BIOLOGY

Poor Response of Malignant Melanomas to Chemotherapy Is Linked to Melanosomes

Chen KG, Valencia JC, Lai B, Zhang G, Paterson JK, Rouzaud F, Berens W, Wincovitch SM, Garfield SH, Leapman RD, Hearing VJ, and Gottesman MM. Melanosomal sequestration of cytotoxic drugs contributes to the intractability of malignant melanomas. *Proc Natl Acad Sci U S A* 103: 9903–7, 2006.

Malignant melanomas are notorious for their resistance to treatments such as radiation and chemotherapy. According to the American Cancer Society, approximately 62,000 new melanoma cases were diagnosed in the United States in 2006, and 7,900 people died of this disease, the fifth most deadly of American cancers. Until now, the precise mechanisms that underlie therapeutic resistance in melanomas remained elusive. More researchers are currently focusing on new, promising therapeutic approaches, such as immunochemotherapy, in an attempt to improve the survival rate of patients with the disease.

Clearly, determining the predominant drug resistance mechanisms is a key step in developing effective therapies. The major cellular/structural difference between melanoma and non-melanoma cancer cells lies in a cytoplasmic organelle called the melanosome. Melanosomes are unique membrane-bound compartments adapted for melanin synthesis in pigment-producing cells, including melanocytes and melanoma cells. Melanosomes also store toxic intermediates produced during melanin synthesis. In this study, we examined the role of melanosomes in drug resistance by directly comparing the melanosomal sequestration of cytotoxic drugs such as *cis*-diaminedichloroplatinum II (CDDP) in MNT-1 melanoma cells and in KB-3-1 epidermoid carcinoma cells.

We initially observed intracellular accumulation of a fluorescent dye (Alexa-Fluor)-labeled platinum compound (designated as AF-CP) in the cytoplasm, but not in the nuclei, of MNT-1 cells. In contrast, KB-3-1 cells accumulated significant amounts of AF-CP in both the cytoplasm and the nuclei. Using immunofluorescence confocal analysis, we colocalized AF-CP with a stage II melanosome marker (i.e., HMB-45) in melanosomes. In our previous study (Liang XJ et al. *J Cell Physiol* 202: 635–41, 2005), we also found that AF-CP reflects at least some biological properties of unmodified CDDP. Thus, we reasoned that the cytoplasmic/melanosomal trapping of AF-CP in melanoma cells likely reflects some properties of chemotherapeutic drugs such as CDDP used in the treatment of melanoma.

To verify the results obtained from the experiments with AF-CP, we used an X-ray probe to directly map the intracellular retention of the platinum compound (which for study purposes

we regarded as unmodified CDDP) both in MNT-1 cells and in KB-3-1 cells. We found that the nuclear retention of CDDP in MNT-1 cells was much less than that which we observed in KB-3-1 control cells. The melanosomal localization of CDDP was also confirmed by melanosomal emission spectrum analysis of platinum. Hence, we were able to colocalize more than 50% of CDDP within melanosomes. These data indicate that the platinum-containing compounds are trapped mainly in subcellular organelles such as melanosomes. Our data thus suggest a fundamental difference between melanoma and nonmelanoma cells in terms of their cytoplasmic/melanosomal and nuclear drug distributions. Clearly, this difference could explain differential chemosensitivity in *in vitro* cellular models and perhaps in melanoma patients.

We further found that melanosome biogenesis could influence the melanosomal localization of AF-CP. In particular, an increase in the generation of stage II or stage II–III melanosomes, but not stage IV melanosomes (the highly pigmented organelles), might significantly change melanosomal drug trapping. Moreover, melanosome biogenesis can be enhanced by various anticancer drugs such as CDDP and vinblastine, which possess different modes of action on their cellular targets. Cytotoxic drug treatment of melanoma cells also caused elevated pigmentation and accelerated melanosome export. Since melanocytes are biologically primed to extrude melanosomes as part of the skin pigmentation process, we speculate that extrusion of drug-containing melanosomes by melanomas also contributes to their relative drug resistance. We found no correlation between melanin content and drug resistance among melanoma cell lines, suggesting that it is the melanosomes per se and not their content of melanin that mediates resistance. We are currently exploring the role of transport systems in enhancing accumulation of cytotoxic drugs in melanosomes as the first step in this novel drug resistance process.

In summary, our studies indicate that melanosome numbers, melanosomal trapping, and melanosome export are involved in drug resistance in melanoma (Figure 1). Cytotoxic drugs such as CDDP can be trapped in subcellular organelles such as melanosomes, thus reducing the drugs' cytotoxicity. Moreover, some cytotoxic drugs can also regulate melanosome numbers as well as pigmentation, which in turn enhances melanosome-mediated drug trapping and accelerates melanosome export. Therefore, our studies provide possible therapeutic approaches to circumventing multidrug resistance in melanomas via inhibition of melanosome biogenesis and melanosome-mediated drug trapping and/or export.

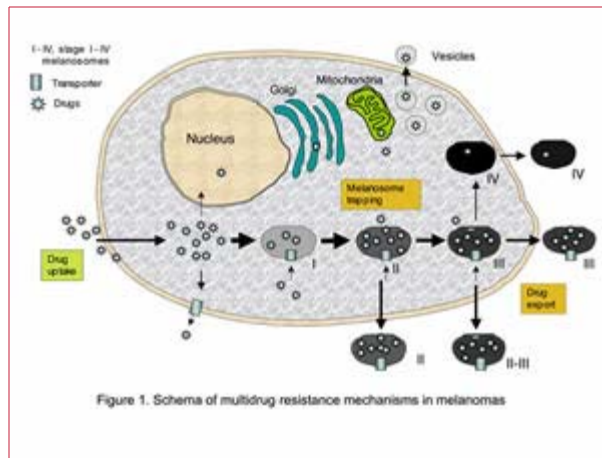


Figure 1. Schema of multidrug resistance mechanisms in melanomas.

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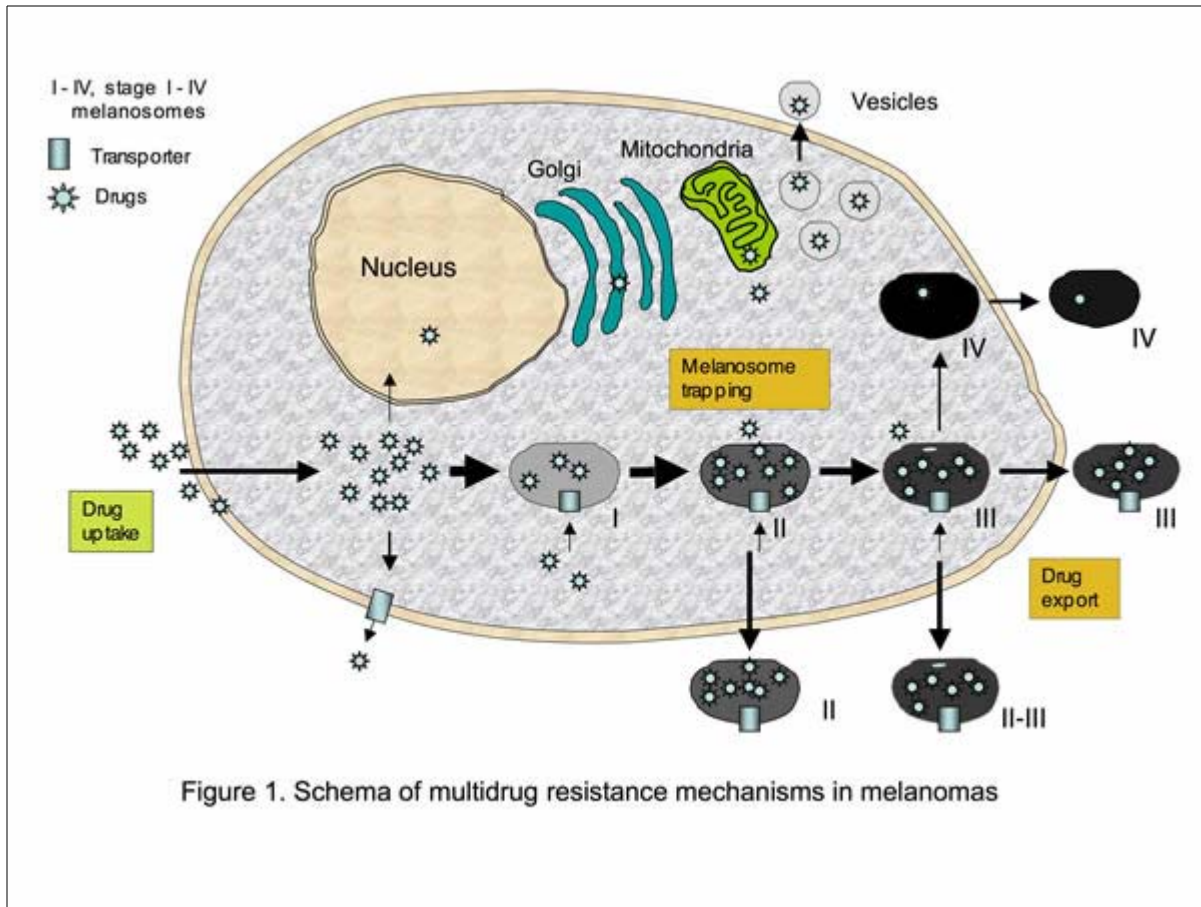


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