Published in final edited form as:
J Invest Dermatol. 2012 October ; 132(10): 2317–2319. doi:10.1038/jid.2012.220.

Beyond ABC: another mechanism of drug resistance in melanoma side population

Mizuho Fukunaga-Kalabis[1] and **Meenhard Herlyn**[1]
[1]Tumormicroenvironment and Metastasis Program, The Wistar Institute, Philadelphia, Pennsylvania, USA

Abstract

It has been shown that a side population (SP), which is characterized by high chemical efflux capacity, is present in human melanoma cell lines. However it was not clear if patients' samples contain the same subpopulation. In this issue, *Luo et al.* (2012), for the first time, isolated SP cells directly from patients' melanomas. SP cells are resistant to paclitaxel due to the upregulation of ABCB1 and ABCB5. Notably, these cells are also resistant to temozolomide, which is not a substrate of ABC transporters, in an IL-8-dependent manner. This study provides novel clues for understanding how this small, but critical, subpopulation within melanomas is resistant to therapies.

Melanomas are often resistant to pharmacologic therapies. Conventional chemotherapy drugs currently used in the clinics are of limited value in the treatment of advanced melanoma. Tumor shrinkage induced by these drugs is often temporary and most tumors progress or relapse after short periods of time. Both intrinsic and acquired mechanisms play roles in the chemoresistance of melanoma cells. One of the key properties for intrinsic resistance is expression of certain ATP-Binding Cassette (ABC) superfamily proteins, which function as ATP-dependent efflux transporters. The ABC family is comprised of nearly 50 members that are evolutionary highly conserved and have high sequence homology among its members. These transporters regulate tissue protection against endogenous and exogenous cellular toxins. Some of the ABC transporters are expressed ubiquitously among diverse cancer cell lines (NCI-60), while others are selectively expressed in cancer cells derived from particular tissue types (Szakacs *et al.*, 2004). Melanoma cells express a group of ABC transporters including ABCA9, ABCB1, ABCB5, ABCB8, ABCC2, and ABCD1 (Chen *et al.*, 2009). Although it has been reported that ABCB5 and ABCB8 mediate doxorubicin resistance in melanoma cells (Elliott and Al-Hajj, 2009; Frank *et al.*, 2005) and ABCC2 expression is associated with cisplatin resistance (Liedert *et al.*, 2003), the physiological functions of most ABC transporters in melanomas have not been elucidated.

The Hoechst 33342 dye exclusion assay is broadly performed to analyze the efflux capacity of cells. When Hoechst 33342 is excited by a UV laser, the dye emits blue (450nm) and red (675nm) wavelengths. A gradient of fluorescence of the dye monitored by flow cytometry at both wavelengths is usually seen as a comma-like region on an x–y plot. The tip of the comma-like region forms a tail, which displays low blue and red fluorescence. This subpopulation, termed `side population' (SP), has high efflux capacity and has been identified in several tissues of mammalian species (Goodell *et al.*, 1996). Likely, the increased efflux capacity of the SP is promoted through ABC transporters. Most commonly,

Correspondence: Meenhard Herlyn, The Wistar Institute, 3601 Spruce Street, Philadelphia, Pennsylvania 19104, USA. herlynm@wistar.org.

The authors state no conflict of interest.

ABCB1 and ABCG2 are highly expressed in SPs of many tissue types and are responsible for transporting Hoechst 33342 as well as chemotherapeutic drugs; vinblastin and paclitaxel are expelled by ABCB1, while topotecan and methotrexate are the substrates of ABCG2 (Hadnagy *et al.*, 2006).

The SP assay initially attracted attention among stem cell researchers as a strategy to isolate potential stem/progenitor cells from various tissues because cell surface markers for stem cells are still not defined in many organ systems. More recently, the assay has been used with cell lines and patient-derived tumor material from various types of cancer to identify cells that exhibit stem cell-like properties. Several groups have characterized SP cells in melanoma (Fukunaga-Kalabis *et al.*, 2010; Grichnik *et al.*, 2006; Roesch *et al.*, 2010; Wouters *et al.*, 2012). Melanoma SPs from cell lines are small in size (Grichnik *et al.*, 2006), slow-growing (Grichnik *et al.*, 2006; Roesch *et al.*, 2010) and, most importantly, resistant to chemotherapy (Fukunaga-Kalabis *et al.*, 2010; Wouters *et al.*, 2012). Although these studies with cell lines suggest that melanomas contain a subpopulation intrinsically resistant to chemotherapeutic drugs, it remains unclear whether the SP phenotype is clinically relevant or more a culture artifact.

In this issue, Luo et al. (Luo *et al.*, 2012) report that patients' melanomas indeed contain a SP. They performed the Hoechst assay on suspensions of melanoma cells, which were directly isolated from tumor tissues and patient-derived tumor xenografts (PDX model). Original tumors showed the presence of SP cells ranged from 0.1 to 0.7% regardless of whether the lesions were primary or metastatic. Melanoma cell lines from previous and the current studies showed a heightened fraction of SP cells, close to 10% of the total population. It is likely that *in vitro* culture systems provide a selective pressure for the expansion of specific tumor subpopulations. PDX tumors showed a similar % of SP cells compared to their original tumor, indicating that this *in vivo* model has the significant advantage of maintaining the heterogeneity of the tumors of origin. Both paclitaxel and temozolomide treatment, the former is the substrate of ABCB1 and the latter is not, increased the ratio of SP cells in the remaining tumors of PDX mice, suggesting that SP cells in melanoma are resistant to chemotherapy *in vivo*. Melanoma SP cells showed higher expression of multiple ABC transporters including ABCB1 and ABCB5 compared to the non-SP fraction. When these two ABC members were knocked down in a melanoma cell line, the percentage of the SP was significantly decreased and the cells regained sensitivity to paclitaxel. Neither knocking down ABCB1 nor ABCB5 affected the response to temozolomide in melanoma. The authors further explored the mechanism responsible for temozolomide resistance of SP cells. The patient-derived SP cells highly expressed IL-8, which has been suggested to be associated with chemoresistance in melanoma cells. Blocking IL-8 signaling with neutralizing antibodies or siRNA significantly increased sensitivity to temozolomide *in vitro* (the former specifically decreased the resistance in a SP). Moreover, microarray data revealed that SP cells are enriched with components of the inflammatory response, especially in the NF-κB signaling pathway, which may contribute to chemoresistance in an ABC transporter-independent manner. These findings suggest that not only efflux capacity, but also other intrinsic mechanisms confer the resistance of melanoma SP cells against multiple drugs, which are not substrates of ABC transporters. Further studies are warranted to determine whether the inhibition of IL-8 or NF-κB signaling can reverse drug resistance of melanoma cells *in vivo*. Such studies will contribute to progress in the therapy of this tumor that remains challenging to treat.

Acknowledgments

This work was supported by grants from the National Cancer Institute (CA 025874 and CA10815).

References

Chen KG, Valencia JC, Gillet JP, et al. Involvement of ABC transporters in melanogenesis and the development of multidrug resistance of melanoma. Pigment Cell Melanoma Res. 2009; 22:740–9. [PubMed: 19725928]

Elliott AM, Al-Hajj MA. ABCB8 mediates doxorubicin resistance in melanoma cells by protecting the mitochondrial genome. Mol Cancer Res. 2009; 7:79–87. [PubMed: 19147539]

Frank NY, Margaryan A, Huang Y, et al. ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. Cancer Res. 2005; 65:4320–33. [PubMed: 15899824]

Fukunaga-Kalabis M, Martinez G, Nguyen TK, et al. Tenascin-C promotes melanoma progression by maintaining the ABCB5-positive side population. Oncogene. 2010; 29:6115–24. [PubMed: 20729912]

Goodell MA, Brose K, Paradis G, et al. Isolation and functional properties of murine hematopoietic stem cells that are replicating in vivo. J Exp Med. 1996; 183:1797–806. [PubMed: 8666936]

Grichnik JM, Burch JA, Schulteis RD, et al. Melanoma, a tumor based on a mutant stem cell? J Invest Dermatol. 2006; 126:142–53. [PubMed: 16417230]

Hadnagy A, Gaboury L, Beaulieu R, et al. SP analysis may be used to identify cancer stem cell populations. Exp Cell Res. 2006; 312:3701–10. [PubMed: 17046749]

Liedert B, Materna V, Schadendorf D, et al. Overexpression of cMOAT (MRP2/ABCC2) is associated with decreased formation of platinum-DNA adducts and decreased G2-arrest in melanoma cells resistant to cisplatin. J Invest Dermatol. 2003; 121:172–6. [PubMed: 12839578]

Luo Y, Ellis L, Dallaglio K, et al. Side Population Cells from Human Melanoma Tumors Reveal Diverse Mechanisms for Chemoresistance. Journal of Investigative Dermatology. 2012

Roesch A, Fukunaga-Kalabis M, Schmidt EC, et al. A temporarily distinct subpopulation of slow-cycling melanoma cells is required for continuous tumor growth. Cell. 2010; 141:583–94. [PubMed: 20478252]

Szakacs G, Annereau JP, Lababidi S, et al. Predicting drug sensitivity and resistance: profiling ABC transporter genes in cancer cells. Cancer Cell. 2004; 6:129–37. [PubMed: 15324696]

Wouters J, Stas M, Govaere O, et al. Gene expression changes in melanoma metastases in response to high-dose chemotherapy during isolated limb perfusion. Pigment Cell Melanoma Res. 2012

Clinical Implications:

- Patient-derived melanoma tissues contain a side population (SP) which is chemoresistant
- ABC transporters ABCB1 and ABCB5 mediate resistance against paclitaxel
- Resistance against temozolomide in SP cells is IL8-dependent