Modern Pathology (2012) 25, 1169–1175

Profiling of ABC transporters ABCB5, ABCF2 and nestin-positive stem cells in nevi, *in situ* and invasive melanoma

Namrata Setia[1,5], Ossama Abbas[2,5], Yessica Sousa[3], Jane L Garb[4] and Meera Mahalingam[3]

[1]*Department of Pathology and Laboratory Medicine, Baystate Medical Center/Tufts University School of Medicine, Springfield, MA, USA;* [2]*Department of Dermatology, American University of Beirut Medical Center, Beirut, Lebanon;* [3]*Department of Dermatology, Dermatopathology Section, Boston University School of Medicine, Boston, MA, USA and* [4]*Department of Epidemiology and Biostatistics, Tufts University School of Medicine, Springfield, MA, USA*

Distinct ABCB5 forms and ABCF2, members of the ATP-binding cassette (ABC) superfamily of transporters, are normally expressed in various tissues and cells, and enhanced expression of both has been demonstrated in select cancers. In melanoma cell lines, gene expression profiling of ABC transporters has revealed enhanced expression of melanocyte-specific ABCB5 and ABCF2 proteins. Given this, our primary aim was to ascertain immunohistochemical expression of the ABC transporters ABCB5 and ABCF2 and, the stem cell marker, nestin in a spectrum of benign and malignant nevomelanocytic proliferations, including nevi ($n=30$), *in situ* ($n=31$) and invasive ($n=24$) primary cutaneous melanomas to assess their role in the stepwise development of malignancy. In addition, their expression was compared with established melanoma prognosticators to ascertain their utility as independent prognosticators. A semiquantitative scoring system was utilized by deriving a cumulative score (based on percentage positivity cells and intensity of expression) and statistical analyses was carried out using analysis of variance with linear contrasts. Mean cumulative score in nevi, *in situ* and invasive melanoma were as follows: 3.8, 4.4 and 5.3 for ABCB5, respectively ($P<0.005$ for all), and 4.6, 4.6 and 5.3 for nestin, respectively ($P=$ not significant for all). No appreciable expression of ABCF2 was noted in any of the groups. While ulcerated lesions of melanoma demonstrated lower levels of expression of ABCB5 and nestin than non-ulcerated lesions, and nestin expression was lower in lesions with mitoses >1, after controlling for the presence of ulceration and mitotic activity, the expression of both proteins did not significantly correlate with known melanoma prognosticators. The gradual increase in the expression of ABCB5 from benign nevus to *in situ* to invasive melanoma suggests that it plays a role in melanomagenesis. On the basis of our findings, a prospective study with follow-up data is required to ascertain the utility of ABCB5 as a therapeutic target.
Modern Pathology (2012) **25,** 1169–1175; doi:10.1038/modpathol.2012.71; published online 4 May 2012

Keywords: ABCB5; ABCF2; ABC transporters; melanoma; nestin

Initially discovered as chemotherapeutic drug-efflux pumps, ATP-binding cassette (ABC) superfamily of transporters represents the largest family of transmembrane proteins.[1] On the basis of the organization and sequence of the nucleotide-binding domain, this superfamily is classified into seven distinct families (A–G) in humans.[2] Although the conserved nucleotide-binding domains of these transporters drive transport, the more variable transmembrane domains create the translocation pathway, providing safe passage for multiple, different substrates.[1,2] The ABC superfamily normally functions in the ATP-dependent transport of structurally diverse molecules and participate in tissue differentiation and survival in various organ

Correspondence: Professor M Mahalingam, MD, PhD, FRCPath, Department of Dermatology, Dermatopathology Section, Boston University School of Medicine, 609 Albany Street, J-301, Boston, MA 02118, USA.
E-mail: mmahalin@bu.edu
[5]The first two authors contributed equally to this work.
Received 16 December 2011; revised 28 February 2012; accepted 28 February 2012; published online 4 May 2012

systems as well.[1,2] There are 48 *ABC* genes in the human genome.[1] Although the function of only some of these transporters is known, a number of them are implicated in multidrug resistance and are recognized causes for failure of cancer chemotherapy.[1] This is the case of ATP-dependent drug efflux transporter P-glycoprotein (P-gp or ABCB1) whose expression on tumor cells has been identified to be a well-recognized mechanism of cancer multidrug resistance by decreasing intracellular drug accumulation.[1]

The ABC transporter superfamily includes ABCB5 and ABCF2. ABCB5 with its several distinct forms is expressed in various tissues, including testis, mammary tissue, melanocytes and retinal pigmented epithelium, and has also been shown to have enhanced expression in several cancer subtypes, including breast cancer, malignant melanoma, colorectal cancer and hepatocellular carcinoma, when compared with their normal tissue counterpart.[1,3–8] The β form of ABCB5 (ABCB5.a) specifically is a half-transporter that has been shown to be expressed in melanocytes and melanoma.[1] It has been shown to be a determinant of membrane potential and regulator of cell fusion in physiological skin progenitor cells.[9] In melanoma, gene expression profiling of ABC transporters has revealed a high mRNA expression of melanocyte-specific ABCB5 and ABCF2 in melanoma cell lines.[10] Furthermore, ABCB5 has recently been shown to be highly expressed in a melanoma stem cell-enriched population, especially CD133+ melanoma stem cells.[3] These ABCB5+ melanoma stem cells were associated with melanoma progression and believed to mediate resistance to doxorubicin.[3] Ablation of this melanoma stem cell population through ABCB5 has been shown to inhibit tumor initiation and growth in preclinical xenotransplantation models, suggesting that it might be of clinical utility as a potential therapeutic target.[3]

Nestin, an intermediate filament protein expressed in the cytoplasm of neuroepithelial cells, is known to be a marker for neural progenitor cells in the central nervous system.[11–14] Recently described as a melanocytic stem cell marker,[12] nestin has been shown to have a significantly higher expression in melanomas compared with nevi and in metastatic melanomas compared with primary melanomas.[12–14]

The primary aim of this study was to ascertain immunohistochemical expression of the ABC transporters ABCB5 and ABCF2 and, the stem cell marker, nestin in a spectrum of benign and malignant nevomelanocytic proliferations that ranged from banal nevi to invasive primary cutaneous melanomas to assess whether they played a role in the development of malignancy. In addition, expression of these markers was compared with established melanoma prognosticators to ascertain their utility as independent prognosticators.

Materials and methods

Study Samples

The study was approved by the Boston University School of Medicine institutional review board (IRB No. H-30441). Archival materials were retrieved from the files of the Skin Pathology Laboratory, Boston University School of Medicine (Boston, MA, USA). A total of 85 cases fixed with formalin and embedded in paraffin were chosen. These included cases of primary cutaneous malignant melanoma ($n=24$), malignant melanoma *in situ* ($n=31$) and benign nevi ($n=30$), all from chronic/intermittent sun-exposed sites. Histological sections of all cases were reviewed and the diagnoses confirmed by the dermatopathologist (MM). All patient data were de-identified. Data regarding patient demographics (patient age and gender) and histopathology (melanoma prognostic factors, including Clark's level, Breslow's thickness, mitoses, ulceration, lymphovascular invasion and regression) were extracted from patient's medical records (Table 1).

Immunohistochemistry

Sections were deparaffinized and peroxidase quenched with 0.3% H_2O_2 for 5 min. Antigen retrieval was performed in a steamer containing pre-heated antigen retrieval buffer solution. Following washing with Tween-20, sections were incubated with rabbit polyclonal antibody for 30, 60 and 20 min for ABCB5, ABCF2 and nestin, respectively, and washed in Tween-20. Dual-link HRP polymer was applied for 30 min and sections were again washed with Tween-20. The color was developed by 5 min incubation with DAB solution and sections were counterstained with hematoxylin. Primary antibodies against ABCB5 (rabbit affinity isolated polyclonal antibody; dilution 1:160; Sigma-Aldrich, St Louis, MO, USA), ABCBF2 (rabbit affinity isolated polyclonal antibody; dilution 1:400; Sigma-Aldrich) and nestin (MAB; dilution 1:100; Millipore, Billerica, MA, USA) were performed. Appropriate positive and negative controls were included. Normal prostate tissue with strong granular cytoplasmic staining served as the positive control for ABCB5 antibody, normal pancreatic tissue sections with strong cytoplasmic and membranous staining of islet cells and acinar cells served as the positive control for ABCF2 and normal skin with cytoplasmic expression in endothelial cells and bulge area of the hair follicle served as the positive control for nestin.

Immunohistochemical expression was evaluated with a semiquantitative scoring system by percentage of positive cells and intensity and using the following scale for percentage positivity: 0 = <10%, 1+ = 10–25%, 2+ = 26–50%, 3+ = 51–75% and 4+ = >76–100%; and the following scale for intensity: 1+ = weak/equivalent to the staining of epi-

Table 1 Demographics for cases of malignant melanoma in study

Cases	*Age (years)*	*Gender*	*Thickness (mm)*	*Mitoses (*n*/10 hpf, 1.25 mm²)*	*Anatomic level*	*Radial growth phase*	*Vertical growth phase*	*Ulceration*	*Regression*	*LVI*
1	29	F	0.80	0	III/IV	Present	Present	Absent	Present	Absent
2	75	M	0.22	>1	IV	Absent	Present	Absent	Absent	Absent
3	56	M	0.51	0	IV	Absent	Present	Absent	Absent	Absent
4	63	F	0.32	0	III	Present	Present	Absent	Present	Absent
5	45	F	0.32	0	II	Absent	Absent	Absent	Absent	Absent
6	50	F	0.47	0	II	Absent	Absent	Absent	Absent	Absent
7	71	M	0.35	0	II	Absent	Absent	Absent	Present	Absent
8	56	M	0.47	>1	IV	Absent	Present	Present	Absent	Absent
9	52	M	0.53	0	II	Absent	Absent	Absent	Absent	Absent
10	62	F	0.39	0	II	Absent	Absent	Absent	Present	Absent
11	70	M	0.56	0	IV	Absent	Present	Absent	Absent	Absent
12	63	F	1.10	>1	IV	Absent	Present	Absent	Absent	Absent
13	52	M	1.20	>1	IV	Present	Present	Absent	Present	Absent
14	69	F	1.20	>1	IV	Present	Present	Absent	Absent	Absent
15	94	M	2.46	>1	IV	Present	Present	Present	Absent	Present
16	50	F	2.40	>1	IV	Absent	Present	Present	Absent	Present
17	67	M	1.20	>1	IV	Present	Present	Present	Present	Absent
18	79	F	0.72	>1	IV	Present	Present	Absent	Absent	Present
19	71	M	0.88	>1	IV	Present	Present	Absent	Absent	Absent
20	44	F	0.84	0	II	Absent	Absent	Absent	Absent	Absent
21	56	M	0.32	0	II	Absent	Absent	Absent	Present	Absent
22	79	F	4.95	>1	IV	Absent	Present	Present	Absent	Present
23	78	F	0.84	>1	IV	Absent	Present	Absent	Present	Absent
24	48	F	0.60	>1	IV	Present	Present	Absent	Absent	Absent

LVI, lymphovascular invasion.

dermis, 2+ = moderate staining and 3+ = strong staining. Proportion scoring was performed only if intensity of tumor cell staining was more than that of overlying epidermis. Histological sections of all cases were reviewed by two board-certified dermatopathologists (initial sign-out on all by a dermatopathologist; cases were then re-reviewed and the diagnoses confirmed by the senior author). All stained slides were initially reviewed and scored by the first author (NS) and re-reviewed by the dermatopathologist (MM) in a blinded manner with respect to diagnosis to ensure consistency of interpretation.

Statistical Analysis

The Pearson correlation coefficient was used to assess the association of ABCB5 and nestin levels in each of the three types of lesions. Mean expression levels for ABCB5 and nestin were compared among lesion types using one-way ANOVA with linear contrasts (for malignant melanoma *in situ* and malignant melanoma groups combined compared with nevi and malignant melanoma *in situ* compared with malignant melanoma.

Results

ABCB5

Positive staining for ABCB5 staining was considered by ascertaining cytoplasmic expression and any nuclear staining was considered background artifact. In normal skin, weak ABCB5 cytoplasmic expression was observed in keratinocytes in the uninvolved epidermis, endothelial cells, eccrine glands, smooth muscle of vessel wall, bulb of hair follicle, arrector piloris muscle and nuclear staining of sebocytes, which served as positive internal controls in each case where they could be visualized.

Mean cumulative score (percentage of positive cells staining + intensity of staining) for ABCB5 expression based on lesion type was as follows: 5.3 (s.d. 1.9) for malignant melanoma, 4.4 (s.d. 0.6) for malignant melanoma *in situ* and 3.8 (s.d. 0.6) for benign nevi (Figures 1 and 2). Differences achieved statistical significance in between all three groups (analysis of variance (ANOVA) showed $P = 0.0047$), between melanoma (malignant melanoma *in situ* and malignant melanoma together) and nevi ($P = 0.0011$), and between malignant melanoma and malignant melanoma *in situ* ($P = 0.05$).

ABCF2

ABCF2 staining was considered positive by ascertaining cytoplasmic expression and any nuclear staining was considered background artifact. In normal skin, weak ABCF2 cytoplasmic expression was observed in the uninvolved epidermis (higher intensity in stratum corneum compared with basal layer), lymphocytes, endothelial cells, eccrine ducts, sebocytes, bulb of hair follicles and perinuclear staining in arrector pili

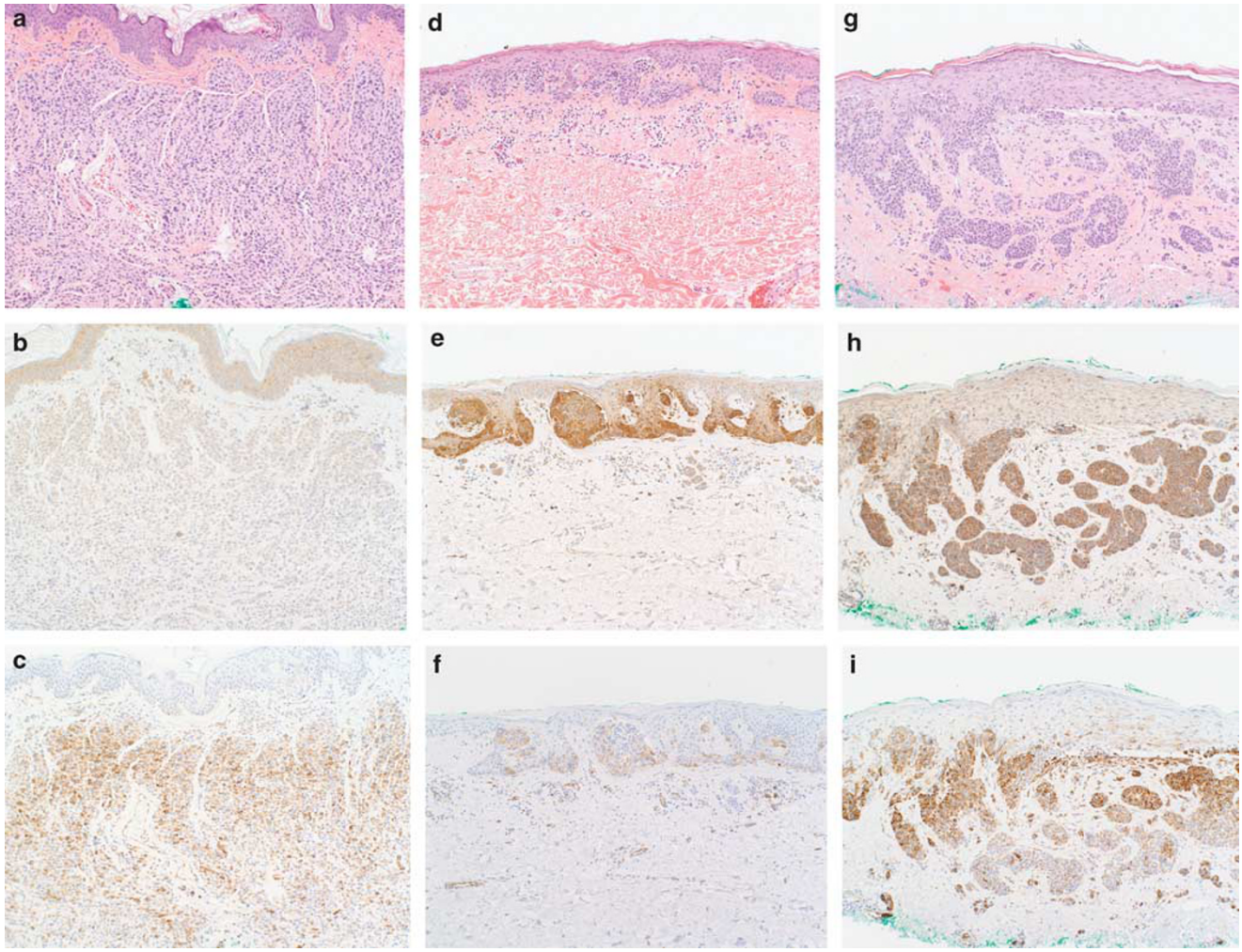

Figure 1 Tumoral expression of ABCB5 and nestin in benign nevi (**a–c**), malignant melanoma *in situ* (**d–f**) and malignant melanoma (**g–i**). **a**, **d**, **g** = hematoxylin and eosin (H&E) (× 10); **b**, **e**, **h** = ABCB5 (× 10); and **c**, **f**, **i** = nestin (× 10). ABC, ATP-binding cassette.

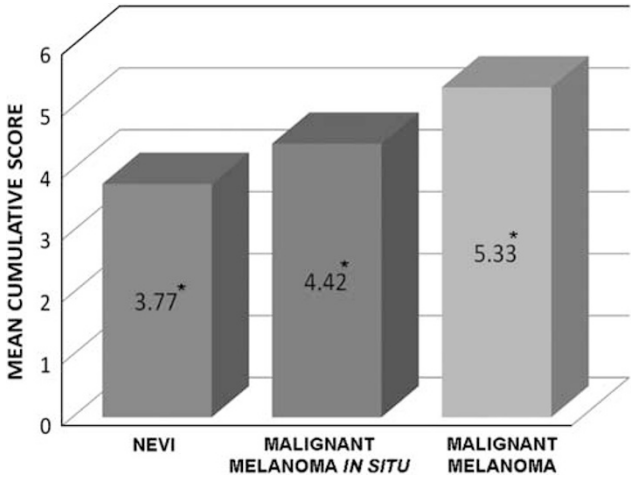

Figure 2 Comparison of mean cumulative score of ABCB5 in malignant melanoma, malignant melanoma *in situ* and benign nevi. *Statistically significant ($P = 0.0047$). ABC, ATP-binding cassette.

muscle, which served as positive internal controls in each case where they could be visualized.

The staining of ABCF2 was either equivalent to or less than that of the epidermis in all three groups. Proportionate staining was to be performed only if the intensity of staining of lesional cells was greater than the epidermis. As a result, the cumulative score for ABCF2 staining was 1+ in all three groups. There was no difference in the pattern/intensity and proportion of the staining in malignant melanoma, malignant melanoma *in situ* and nevi.

Nestin

Nestin staining was also considered positive by ascertaining cytoplasmic expression and any nuclear staining was considered background artifact. In normal skin, nestin expression was observed in endothelial cell cytoplasm and bulge area of the

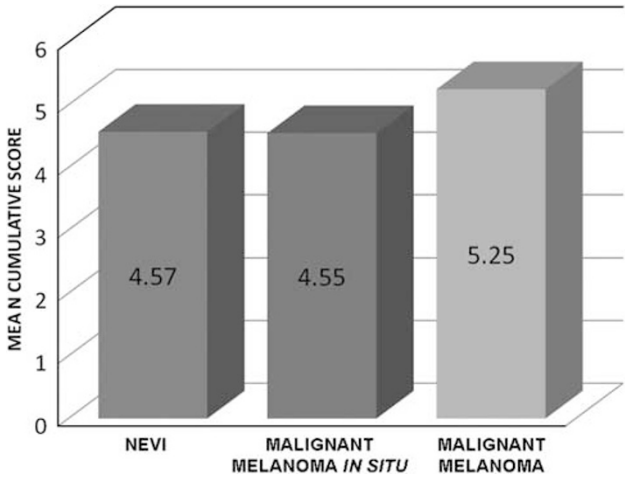

Figure 3 Comparison of mean cumulative score of nestin in malignant melanoma, malignant melanoma *in situ* and benign nevi. P = not significant for all.

Table 2 Correlation of ABCB5 and nestin expression by lesion type

Lesion	R	P-*value*
Malignant melanoma	0.32	0.1289
Malignant melanoma *in situ*	0.49	0.0052
Nevi	0.72	<0.0001

R, correlation co-efficient.

hair follicle, which served as positive internal control in each case where they could be visualized.

Mean cumulative score for nestin expression based on lesion type was as follows: 5.3 (s.d. 0.8) for malignant melanoma, 4.6 (s.d. 0.8) for malignant melanoma *in situ* and 4.6 (s.d. 0.6) for benign nevi (Figures 1 and 3). There was no significant difference among the three groups in mean nestin expression in a one-way ANOVA ($P = 0.3128$).

We also attempted to correlate the expression of ABCB5 and nestin in the different groups (Table 2). There was a significant correlation in their expression in malignant melanoma *in situ* and benign nevi, with a much higher correlation in nevi in comparison with malignant melanoma *in situ*. There was no significant correlation in their expression in malignant melanoma.

Correlation of ABCB5 and Nestin with Known Melanoma Prognosticators

Further, we attempted to correlate the expression of ABCB5 and nestin with age, gender, Clark's level, Breslow's thickness, presence of radial/vertical growth phase, mitoses, ulceration, lymphovascular invasion and regression. In ulcerated lesions, ABCB5 expression was lower than in non-ulcerated lesions (Table 3). After controlling for the presence of ulceration, the expression of ABCB5 did not significantly correlate with any of the known prognostic factors. Likewise, nestin expression was lower in ulcerated and lesions with mitoses >1 ($P < 0.001$ and < 0.026, respectively). After controlling for the presence of ulceration and mitoses, nestin expression also did not significantly correlate with any of the known prognostic factors.

Table 3 Comparison of mean cumulative score of ABCB5 and nestin in ulcerated and non-ulcerated cases of malignant melanoma and in cases of malignant melanoma with mitoses <1 and >1

	Mean ABCB5 expression	P–*value*	*Mean nestin expression*	P-*value*
Ulcerated ($n = 5$)	3.8	0.002	4.4	0.001
Non-ulcerated ($n = 19$)	5.7		5.5	
Mitoses <1 ($n = 11$)	4.9	0.84	4.9	0.027
Mitoses >1 ($n = 13$)	5.5		5.5	

Discussion

A role for ABCB5 in the pathogenesis and development of chemoresistance has been demonstrated previously.[1,3] Findings from this study extend the functionality of ABCB5 to include melanomagenesis as we found significantly increased expression of ABCB5 in *in situ* and invasive melanoma as a group compared with benign nevi. While Sharma *et al*[11] and Schatton *et al*[5] also observed overexpression of ABCB5 in primary melanoma, in terms of primary lesions, both study groups were limited to nevi and melanoma.[5,11] Our observation of a higher level of expression of ABCB5 in invasive compared with *in situ* melanoma and *in situ* melanoma compared with benign nevi indicate that ABCB5 is a key player in the stepwise development of melanoma.

In light of our findings, we then attempted to ascertain the utility of ABCB5 as an independent prognosticator. We found that in patients with invasive melanoma, expression of ABCB5 did not correlate with age, sex, Clark's level, presence of radial/vertical growth phase, mitoses, lymphovascular invasion and regression. In ulcerated lesions, while expression of ABCB5 was lower than in non-ulcerated lesions, after controlling for the presence of ulceration, the expression of ABCB5 did not significantly correlate with any of the known melanoma prognosticators. Although this diminishes the utility of ABCB5 as an independent prognosticator, ulceration is an established, independent prognosticator in primary cutaneous melanoma. Thus, an alteration in expression relative to this parameter

implies biological significance. However, possible limiting factors of this study include the cross-sectional nature and moderately small size of this study set. Thus, biological relevance can only be ascertained in a longitudinal study with an increased number of cases.

From a clinical perspective, the significance of ABCB5 expression appears to be intimately related to drug resistance.[3,9] It has been shown that ABCB5 marks cells with the CD133 immunophenotype, a marker defining progenitor cells among human melanocytes. In the same study, the authors demonstrated that ABCB5 determines as a regulator of membrane potential the propensity of this subpopulation to undergo cell fusion.[9] In physiological progenitor cells, ABCB5 functions to maintain membrane hyperpolarization, associated with multidrug resistance phenotype of cancer cells including melanoma.[3] It is believed that ABCB5 mediates resistance to chemotherapeutic agents such as doxorubicin through drug efflux leading to decreased intracellular drug accumulation.[3] Blocking ABCB5 significantly reverses resistance of melanoma cell lines to doxorubicin, identifying ABCB5 as a novel drug transporter and mediator of chemoresistance in malignant melanoma. A similar role of ABCB5 has also been implicated in other cancers such as hepatocellular carcinoma[8] and colorectal carcinoma.[7] More recently, using serial human-to-mouse xenotransplantation experiments, Schatton *et al*[5] identified a subpopulation of cancer stem cells called malignant-melanoma-initiating cells based on their expression of ABCB5. They established that ABCB5+ cancer cells, comprising 2–20% of the entire tumor cell population, had enhanced tumorigenicity and possessed an exclusive capacity to self-renew and to give rise to more differentiated melanoma cells. More importantly, using tissue microarrays, a positive correlation was demonstrated between neoplastic progression in human melanoma patients and the abundance of this cancer stem cell subset.[5] Using flow cytometric analyses, the same group identified a subpopulation enriched for human malignant-melanoma-initiating cells defined by the expression of ABCB5. Using the technique of suppression subtractive hybridization to investigate the molecular signature of melanoma progression in cell lines, another group found preferential expression of the *ABCB5* gene by metastatic melanomas as opposed to the primary tumor.[15] On the basis of all of this evidence, it appears that ABCB5 in human malignant melanoma provides a unique direct link between cancer stem cells and cancer therapeutic resistance to select chemotherapeutic agents. Our results support a role for ABCB5 in neoplastic progression and the stepwise development of melanoma.[16]

Nestin, an intermediate filament protein expressed in the cytoplasm of neuroepithelial cells and a marker for neural progenitor cells in the central nervous system, has been described as a marker of melanocytic stem cells.[11–14] Previous studies have shown that there is an increased expression of this protein in malignant *vs* benign nevomelanocytic proliferations and that there appears to be a stepwise increase in the proportion of lesions expressing nestin from banal nevi to primary melanomas to metastatic melanoma.[12,13] More recently, nestin staining in stage I and II melanoma patients significantly predicted poor survival with lower rates in cases with nestin positivity in both tumoral and endothelial cells.[14] Consistent with these findings, we observed enhanced expression of nestin in invasive melanoma compared with *in situ* melanoma and nevi, although differences did not quite achieve statistical significance. From a scientific perspective, nestin expression in nevi suggests that it may not be necessarily associated with tissue dedifferentiation and indicates that nestin cannot be used to distinguish benign from malignant melanocytic lesions. We observed that expression of nestin, like that of ABCB5, did not correlate with age, sex, Clark's level, presence of radial/vertical growth phase, mitoses, lymphovascular invasion and regression. In ulcerated lesions and lesion with mitotic count >1, nestin expression was lower than that in non-ulcerated lesions. However, after controlling for the presence of ulceration and mitotic count, nestin expression did not significantly correlate with any of the known prognosticators. Of the three sample groups studied, a significant correlation between expression of ABCB5 and nestin was observed by us in nevi and malignant melanoma *in situ* but not in invasive melanoma, the precise significance of which is not, albeit as yet, clear.

Although it has a structure different from other members as it contains a pair of nucleotide-binding domains without transmembrane domains, ABCF2 is yet another member of the ABC transporter family whose function in cancer cells is still unclear.[17–19] While one study suggested that ABCF2 may contribute to the chemoresistant cancer phenotype as it was overexpressed in chemoresistant clear-cell ovarian carcinoma, another on breast cancer suggested that expression of ABCF2 might indicate good prognosis.[18,19] Recently, Heimrl *et al*[10] observed a striking upregulation of ABCF2 at the transcriptional mRNA level in primary and metastatic melanoma cell lines compared with normal human epidermal primary melanocytes, raising the possibility that it may play a role in the development and progression of malignant melanoma. However, similar findings at the translational level were not replicated in our study as tumor cells in all three groups exhibited equivalent or less protein expression than the epidermis, which, in keeping with the manufacturer's observations, showed very strong ABCF2 staining. Thus, our findings suggest that ABCF2 is not related to the pathogenesis of melanocytic proliferations.

ABCG2 is another ATP transporter expressed in various cancer stem cells lines and has been linked to prognosis, disease-free survival and response to chemotherapy in acute myelogenous leukemia. Its role in chemoresistance has also been studied in breast carcinoma, non-small-cell lung carcinoma, oral squamous cell carcinoma and advanced testicular germ cell tumors.[20] Contradictory results have been found regarding its expression in melanoma cell lines.[21,22] Deichmann *et al*[21] studied 18 melanoma resection specimens and could not demonstrate ABCG2 mRNA upregulation by reverse transcriptase polymerase chain reaction, while Monzani *et al*[22] found *in vitro* and *in vivo* ABCG2 expression in all the cells expressing CD133 by reverse transcriptase polymerase chain reaction and immunohistochemistry.

Although of limited value as an independent prognosticator, in this cross-sectional study the gradual increase in expression of ABCB5 from benign nevus to *in situ* to invasive melanoma suggests that it plays a role in melanomagenesis. On the basis of our findings, a prospective study with follow-up data is required to ascertain the utility of ABCB5 as a therapeutic target.

Disclosure/conflict of interest

The authors declare no conflict of interest.

References

1 Wenzel JJ, Piehler A, Kaminski WE. ABC A-subclass proteins: gatekeepers of cellular phospho- and sphingolipid transport. Front Biosci 2007;12:3177–3193.
2 Moitra K, Scally M, McGee K, *et al.* Molecular evolutionary analysis of ABCB5: the ancestral gene is a full transporter with potentially deleterious single nucleotide polymorphisms. PLoS One 2011;6:e16318.
3 Frank NY, Margaryan A, Huang Y, *et al.* ABCB5-mediated doxorubicin transport and chemoresistance in human malignant melanoma. Cancer Res 2005;65: 4320–4333.
4 Chen KG, Szaka'cs G, Annereau JP, *et al.* Principal expression of two mRNA isoforms (ABCB 5alpha and ABCB5 beta) of the ATP-binding cassette transporter gene ABCB5 in melanoma cells and melanocytes. Pigment Cell Res 2005;18:102–112.
5 Schatton T, Murphy GF, Frank NY, *et al.* Identification of cells initiating human melanomas. Nature 2008; 451:345–349.
6 Arce C, Perez-Plasencia C, Gonzalez-Fierro A, *et al.* A proof-of-principle study of epigenetic therapy added to neoadjuvant doxorubicin cyclophosphamide for locally advanced breast cancer. PLoS One 2006;1:e98.
7 Wilson BJ, Schatton T, Zhan Q, *et al.* ABCB5 identifies a therapy-refractory tumor cell population in colorectal cancer patients. Cancer Res 2011;71:5307–5316.
8 Cheung ST, Cheung PF, Cheng CK, *et al.* Granulin–epithelin precursor and atp-dependent binding cassette ABCB5 regulate liver cancer cell chemoresistance. Gastroenterology 2011;140:344–355.
9 Frank NY, Pendse SS, Lapchak PH, *et al.* Regulation of progenitor cell fusion by ABCB5 P-glycoprotein, a novel human ATP-binding cassette transporter. J Biol Chem 2003;278:47156–47165.
10 Heimerl S, Bosserhoff AK, Langmann T, *et al.* Mapping ATP-binding cassette transporter gene expression profiles in melanocytes and melanoma cells. Melanoma Res 2007;17:265–273.
11 Sharma BK, Manglik V, Elias EG. Immuno-expression of human melanoma stem cell markers in tissues at different stages of the disease. J Surg Res 2010;163: e11–e15.
12 Klein WM, Wu BP, Zhao S, *et al.* Increased expression of stem cell markers in malignant melanoma. Mod Pathol 2007;20:102–107.
13 Brychtova S, Fiuraskova M, Hlobilková A, *et al.* Nestin expression in cutaneous melanomas and melanocytic nevi. J Cutan Pathol 2007;34:370–375.
14 Piras F, Perra MT, Murtas D, *et al.* The stem cell marker nestin predicts poor prognosis in human melanoma. Oncol Rep 2010;23:17–24.
15 Sousa JF, Espreafico EM. Suppression subtractive hybridization profiles of radial growth phase and metastatic melanoma cell lines reveal novel potential targets. BMC Cancer 2008;8:19.
16 Bennett DC. Human melanocyte senescence and melanoma susceptibility genes. Oncogene 2003;22: 3063–3069.
17 Yasui K, Mihara S, Zhao C, *et al.* Alteration in copy numbers of genes as a mechanism for acquired drug resistance. Cancer Res 2004;64:1403–1410.
18 Nishimura S, Tsuda H, Ito K, *et al.* Differential expression of ABCF2 protein among different histologic types of epithelial ovarian cancer and in clear cell adenocarcinomas of different organs. Hum Pathol 2007;38:134–139.
19 Ogawa Y, Tsuda H, Hai E, *et al.* Clinical role of ABCF2 expression in breast cancer. Anticancer Res 2006;26: 1809–1814.
20 An Y, Ongkeko WM. ABCG2: the key to chemoresistance in cancer stem cells? Expert Opin Drug Metab Toxicol 2009;5:1529–1542.
21 Deichmann M, Thorne M, Egner U, *et al.* The chemoresistance gene ABCG2 (MXR/BCRP1/ABCP1) is not expressed in melanomas but in single neuroendocrine carcinomas of the skin. J Cutan Pathol 2005;32:467–473.
22 Monzani E, Facchetti F, Galmozzi E, *et al.* Melanoma contains CD133 and ABCG2 positive cells with enhanced tumourigenic potential. Eur J Cancer 2007; 43:935–946.