

Chapter 13

Prominin-1 (CD133) and Metastatic Melanoma: Current Knowledge and Therapeutic Perspectives

Aurelio Lorico, Javier Mercepide, and Germana Rappa

Abstract Innovative approaches to specifically target the melanoma subpopulation responsible for local invasion and metastatic dissemination are needed. Prominin-1 (CD133) expression has been observed in many melanoma cell lines, as well as in primary and metastatic melanomas from patients. Although its function(s) in melanoma is presently unknown, prominin-1 may represent a molecular target, due to its association with melanoma stem cells and with the metastatic phenotype.

Keywords CD133 • Melanoma • Immunotherapy • Stem cells • AC133 • Wnt

13.1 Introduction

The incidence of melanoma is steadily increasing in Caucasian populations worldwide, with approximately 70,000 new cases per year in the United States alone [1]. Of all deaths associated with any type of skin cancer, about 85% are caused by melanoma, and the development of metastasis is by far the major cause of melanoma deaths [1]. Metastatic melanoma is largely refractory to all currently available systemic therapies, with infrequent durable responses to conventional chemotherapy,

A. Lorico (✉) • G. Rappa
Cancer Research Program, Roseman University of Health Sciences,
89104 Henderson, NV, USA

College of Pharmacy, Roseman University of Health Sciences,
89104 Henderson, NV, USA
e-mail: alorico@roseman.edu

J. Mercepide
Cancer Research Program, Roseman University of Health Sciences,
89104 Henderson, NV, USA

immunotherapy, or to the recent BRAF (v-raf homolog B1) inhibitors [2]. Potentially, metastatic cells represent a minority of the bulk population of melanoma cells that must be specifically targeted to prevent metastatic dissemination. Many studies have identified expression of prominin-1 (CD133) in both established melanoma cell lines and clinical specimens derived from melanoma patients [3–5], generally by immunoreactivity for the glycosylation-dependent monoclonal antibody (mAb) CD133/1 (clone AC133; Miltenyi Biotec, Bergisch Gladbach, Germany). Our laboratory showed that prominin-1 knockdown in human FEMX-I metastatic melanoma cells resulted in decreased metastatic potential [6]. Prominin-1, normally expressed on undifferentiated cells, including hematopoietic stem cells, endothelial progenitor cells, fetal brain stem cells, and prostate epithelial cells, has also been exploited to identify and purify cancer stem cells (CSC) from various solid tumors including brain, colon, prostate, and pancreatic cancers [7]. Thus, despite its unknown function in stem cell biology, prominin-1 appears to mark both normal and CSC. In this chapter, we will summarize the existing literature data on the expression of prominin-1 in melanoma and in melanoma stem cells (MSC) and its association with the metastatic phenotype, and we will examine its potential role as a therapeutic target for melanoma.

13.2 Melanoma Development

Two, probably coexisting, models of melanoma development have been proposed:

1. Normal mature melanocytes acquire progressive mutations, which lead the cells through the classical phases of benign nevus, dysplastic nevus, and radial and vertical growth phases, ultimately resulting in metastatic melanoma.
2. Melanomas arise from the transformation of neural crest-derived melanocytic stem or immature progenitor cells [8], resulting directly in local growth, followed by invasion and metastatic colonization. Interestingly, prominin-1 is expressed on the surface of dermal-derived stem cells that are capable of differentiating into neural cells [9].

In both developmental models, an important role of the tumor microenvironment, including inflammatory, mesenchymal, and endothelial cells in the invasive/metastatic process, has been postulated. The observation that the majority of melanomas do not evolve from melanocytic proliferations, such as nevi [10], is in favor of the clinical relevance of the stem cell developmental model [11].

13.3 Melanoma Stem Cells

The definition of MSC, as for all CSC, is at present only functional, encompassing the capacities to initiate melanomas when implanted into immunodeficient animals, to reconstitute the heterogeneity of the original tumor, and to successfully establish

tumors upon serial transplantation. While self-renewing MSC maintain the stem cell compartment, they also give rise to progenitor cells with limited self-renewing capacity and ultimately to differentiating clones of varying dominance, which contribute to the cell heterogeneity typically found in melanomas. The MSC model does not address the question of whether melanomas arise from normal melanocyte stem cells. Rather, it suggests that, irrespective of the cell of origin, melanomas are hierarchically organized, with MSC undergoing epigenetic changes analogous to the differentiation of normal cells, forming phenotypically diverse non-tumorigenic cancer cells that make up the bulk of the tumor.

For clinical applications, molecules present on the surface of MSC would allow their diagnostic identification in melanoma biopsies and might be used as prognostic indicators and therapeutic targets. In fact, while most treatment strategies are currently aimed at the bulk tumor population, MSC surface molecules may constitute targets for antibody-based or other types of therapies that would selectively eliminate these cells.

The notorious resistance of melanoma cells to all major chemotherapeutic drugs strongly suggests that this tumor type possesses major properties for drug resistance, presumably intrinsic to the MSC subpopulation. Without targeting the minor hidden fraction of partially quiescent or drug-resistant MSC, the goal of curing this disease may be unattainable, with anti-melanoma regimens, resulting in melanoma regrowth.

Cells with stem cell markers and features have recently been identified in melanoma tissues and cell lines. While several of these proteins, including prominin-1, CD271, CD20, nestin, ABCB5, and Bmi-1 have been proposed as MSC markers [3–5, 12–14], none of them have so far been shown to conclusively identify the MSC subpopulation [15]. It has been suggested that the use of nonobese diabetic mice with severe combined immunodeficiency disease (NOD/SCID mice) in xenotransplantation experiments can underestimate the frequency of human cancer cells with tumorigenic potential due to the xenogeneic immune response in these mice [16]. Thus, although only ~1 in a million melanoma cells reportedly formed tumors in NOD/SCID mice, 1 in 4 could form tumors in NOD/SCID IL2R γ^{null} mice when coinjected with Matrigel, a substance mimicking the basement membrane. The possibility has been raised that some melanomas follow a cancer stem cell model, whereas others do not [16].

Melanoma side population (SP), a functional subpopulation distinct by the capacity to efflux a Hoechst dye with great efficiency, has also been proposed to contain the putative MSC. Interestingly, the major representative of the SP phenotype, the ABC transporter ABCG2 [17], has also been shown to be co-expressed on a subpopulation of prominin-1⁺ melanoma cells [5]. In another study, melanoma cells expressing ABCB5, another energy-dependent drug efflux transporter highly similar to ABCB1, highly co-expressed prominin-1 and could enrich for prominin-1⁺ cells, indicating that ABCB5⁺/prominin-1⁺ may mark MSC [4].

Whether prominin-1⁺, CD271⁺, and any other putative MSC subpopulation isolated from cultures or from clinical specimens, based on surface markers, overlap or are indeed the true tumor-initiating cells requires further characterization, and how these correlate with clinical outcome remains to be determined.

13.4 Expression of Prominin-1 in Established Human Melanoma Cell Lines

Several established melanoma cell lines express prominin-1, generally at relatively high levels and in the great majority of cells. Gil-Benso and colleagues [18] recently established the MEL-RC08 metastatic melanoma cell line with mutations in both BRAF and TP53 genes, derived from a pericranial metastasis of a malignant melanoma of the skin. A high percentage (84.1%) of MEL-RC08 cells expressed prominin-1. Four clonal cell lines obtained from the parental MEL-RC08 cell line also showed a high percentage of prominin-1⁺ cells, ranging from 87% to 99%.

In a separate study, Monzani and colleagues investigated [5] prominin-1 expression in four independently derived human melanoma cell lines, namely, WM115, A375, IGR 32, and IGR 39. In all cases, practically all cells expressed prominin-1. In particular, for WM115 cells, prominin-1 expression was observed in all cells by means of fluorescent-activated cell-sorting (FACS) analysis, immunofluorescence, and reverse transcriptase-polymerase chain reaction (RT-PCR). WM115 expressed both prominin-1 and ABCG2, another putative MSC marker (see above). This cell line grows as floating spheroids, expresses typical progenitor and mature neuronal/oligodendrocyte markers, and is able to transdifferentiate into astrocytes or mesenchymal lineages under specific growth conditions. In WM115 melanoma xenografts, prominin-1 levels significantly decreased in tumors propagated in mice; yet on *in vitro* reculturing, cells readily regained high levels of prominin-1, suggesting that *in vivo* tumor growth conditions can significantly differ from *in vitro* culture conditions and can influence observed expression patterns [5].

Pietra and colleagues reported that five out of eight melanoma cell lines were negative for prominin-1, while in two (i.e., MeTA and Me1386), essentially all cells were prominin-1⁺, and one (i.e., FO-1) displayed a bimodal distribution of prominin-1 on the cell surface [19].

Our laboratory, employing the AC133 mAb, found that essentially all human FEMX-I metastatic melanoma cells in culture expressed prominin-1 on their surface, with an average 400,000 molecules/cell [6]. As described later, while *in vitro*, a high percentage of cells expressing prominin-1 is generally observed, in freshly isolated melanoma cells from patients, only a minor subpopulation of prominin-1⁺ cells (<1%) is present.

Combined, these studies establish the expression of prominin-1 in melanomas, supporting the notion that melanomas contain a stem cell component. However, comprehensive studies to correlate prominin-1 expression to self-renewal, differentiation, and tumorigenicity remain to be conducted.

13.5 Prominin-1 in Experimental Metastasis Models

Kim and colleagues [20] showed that administration of dacarbazine enriched a distinct prominin-1⁺ subpopulation of B16F10 murine melanoma, with enhanced metastatic potential *in vivo*. Prominin-1⁺ tumor cells were located close to tumor-associated

lymphatic vessels in metastatic organs. Lymphatic vessels in metastatic tissues stimulated chemokine receptor 4 (CXCR4)⁺/prominin-1⁺ cell metastasis to target organs by secretion of stromal cell-derived factor-1 (SDF-1, CXCL12), a ligand of CXCR4. The authors suggested that targeting the SDF-1/CXCR4 axis in addition to dacarbazine treatment could therapeutically block chemoresistant prominin-1⁺ cell metastasis toward a lymphatic metastatic niche.

In a study published by our laboratory [6], downregulation of prominin-1 had profound effects on human FEMX-I metastatic melanoma cells, including slower cell growth, reduced cell motility, and decreased capacity to form spheroids under stem cell-like growth conditions. Also, prominin-1 downregulation severely reduced the metastatic capacity of melanoma tumor xenografts, particularly to lung and spinal cord. These data strongly suggest that prominin-1 plays an important role in the melanoma cells' capacity to seed to distant sites. By microarray analysis, we also observed that many genes upregulated in prominin-1-knockdown cells coded for Wnt inhibitors, suggesting an interaction between prominin-1 and the canonical Wnt pathway [6]. In a subsequent study, our laboratory reported that three distinct pools of prominin-1 coexisted in cultures of FEMX-I cells. Morphologically, in addition to the plasma membrane localization, prominin-1 was found within the intracellular compartments (e.g., Golgi apparatus) and in association with extracellular membrane vesicles, most likely exosomes (unpublished observations). Since the function of microvesicles is dependent on the molecular cargo that they carry (e.g., protein, RNAs), the observation that microvesicles released by metastatic melanoma cells contain prominin-1 suggests that this molecule may have a certain role in extracellular communication.

Degradation of the basement membrane is a critical step in promoting tumor metastasis. We found that prominin-1 knockdown results in reduced cell motility and misalignment, presumably because cells could not organize properly due to impaired migration. We also observed loss of their capacity to invade through Matrigel, a basement membrane-mimicking substance [6]. Interestingly, it was previously shown that tumor-derived microvesicles often carry protein-degrading enzymes, including matrix metalloproteinases. By releasing them, tumor cells can degrade the extracellular matrix and invade surrounding tissues. Functionally, the downregulation of prominin-1 in FEMX-I cells resulted in upregulation of Wnt inhibitor genes [6] and decrease in the nuclear localization of β -catenin, a surrogate marker of Wnt activation. Moreover, the T cell factor/lymphoid enhancer factor (TCF/LEF) promoter activity was higher in parental than in prominin-1-knockdown cells (unpublished observations). A constitutive complex of β -catenin and LEF-1 was detected in melanoma cell lines, expressing either mutant β -catenin or mutant adenomatous polyposis coli tumor suppressor protein (APC) [21]; however, β -catenin mutations are rare in primary malignant melanoma, while its nuclear localization, a potential indicator of Wnt/ β -catenin pathway activation, is frequently observed in melanoma [22]. The relevance of Wnt signaling to the metastatic process has only recently been unveiled by the observation that β -catenin is a central mediator of melanoma metastasis to the lymph nodes and lungs [23]. Interestingly, a clear association between prominin-1 and nuclear β -catenin exists for colon

carcinoma [24]. It is conceivable, as recently proposed by Boivin and colleagues [25], that phosphorylation of prominin-1 in response to an unknown extracellular ligand might activate the Wnt pathway that would influence cell growth, motility, and metastatic potential. Also, the presence of conserved tandem TCF/LEF binding sites in the *prominin-1* (*Prom1*) gene suggests a feedback regulation of prominin-1 expression by the Wnt pathway [26]. Prominin-1-mediated Wnt activation, probably linked to the dynamic organization of cholesterol-rich membrane microdomains, may be responsible for the precise coordination and integration of the expression of multiple genes, resulting in dynamic, pro-metastatic changes of cell adhesion, and motility. Collectively, our recent results pointed to Wnt signaling and/or release of prominin-1-containing membrane vesicles as mediators of the pro-metastatic activity of prominin-1 in FEMX-I melanoma.

13.6 Expression of Prominin-1 in Patient-Derived Primary and Metastatic Melanoma Specimens

Surprisingly, little data are present in the literature on prominin-1 expression in primary melanomas, sentinel lymph nodes, and distant metastasis. Studies on the presence of distinct intracellular pools of prominin-1 have neither been performed nor has the reactivity of clinical specimens with different anti-prominin-1 mAbs been investigated. Monzani and colleagues determined that less than 1% of cells within metastatic melanomas expressed prominin-1 [5]. Only prominin-1⁺ cells collected from these biopsies induced tumors in NOD/SCID mice, whereas the prominin-1⁻ fraction failed to regenerate tumors, indicating that only a small percentage of cells was capable of recapitulating tumor growth [5]. Klein and colleagues [3] observed by immunohistochemistry significant increases in the expression of prominin-1 as well as of CD166 and nestin in primary and metastatic melanomas compared with banal nevi. Increased melanoma aggressiveness corresponded with greater expression of these markers, suggesting that during melanoma progression, stem cell markers become more evident, probably due to an increase of the dysregulation of stem/progenitor cell function and proliferation. However, in that study, only nestin showed a statistical difference when comparing primary and metastatic melanoma [3]. In 30 cases of lentigo maligna melanoma, analyzed by immunohistochemical staining, Bongiorno and colleagues [27] found in the vast majority of cases the presence of prominin-1⁺ cells in the outer root sheath of the mid-lower hair follicles, intermixed with atypical melanocytes extending along layers of the hair follicles. While the authors concluded that this finding supports the origin of melanoma from melanocyte stem cells, no conclusion on the expression of prominin-1 in lentigo maligna melanoma cells was possible. Piras and colleagues [28] also analyzed prominin-1 expression by immunohistochemistry in 130 primary melanomas and 32 nodal metastasis biopsy specimens. Therein, prominin-1 staining was neither associated with survival, nor significant differences in prominin-1 expression were observed between primary tumors and metastases. Employing RT-PCR, Gazzaniga and colleagues [29] detected

prominin-1⁺ cells in the sentinel lymph nodes from 18 out of 45 total patients with stage I, II, and III. However, presumably for the low number of patients analyzed, no statistically significant correlations between prominin-1 expression and overall survival were found.

In a recent study [30], patient-derived melanoma cells could be maintained in cell culture for more than 16 months as melanospheres in serum-free cultures. The transition from melanospheres to monolayers was accompanied by an apparent loss of clonogenic potential and reversible changes in expression of cell surface markers, including prominin-1. Compared with adherent monolayer cultures, melanospheres were enriched in cells with clonogenic potential, reflecting the self-renewing capacity of cancer stem-like cells. The authors concluded that melanoma cells easily change their function upon exposure to external stimuli and suggested that the frequency of melanoma stem-like cells strongly depends on the microenvironment.

In a report by Fusi and colleagues [31], in about 50% of patients affected by metastatic melanoma, a small fraction of circulating melanoma cells isolated from peripheral blood expressed prominin-1. Its expression was not associated with a shorter overall survival. However, tissue positivity for prominin-1 was detected by immunohistochemistry in all patients with accessible metastases [31]. In support of a role of prominin-1 in the metastatic process, Thill and colleagues [32] found by immunohistochemistry the presence of prominin-1⁺ cells in uveal melanomas, predominantly at the invading tumor front. Further evidence of association of prominin-1 with the metastatic potential stems from an analysis by Kupas and colleagues [33], showing that melanoma cells expressing the receptor activator of NF- κ B (RANK) co-expressed prominin-1 and were able to induce tumor growth in immunodeficient mice. The RANK-RANK ligand pathway, involved in the migration and metastasis of epithelial tumor cells [33], increased significantly in peripheral circulating melanoma cells, primary melanomas, and metastases from stage IV melanoma patients compared with tumor cells from stage I melanoma patients. Also, a statistically significant overexpression of prominin-1 in 15 patients with lymph node metastasis and 19 patients with distant metastases compared to benign nevi was observed by Sharma and colleagues [34] (Table 13.1).

13.7 Therapeutic Perspectives

Since CSC in general have been reported to be both drug and radioresistant [35, 36], it is generally accepted that novel therapeutic approaches are urged to eradicate the malignant melanoma clone(s). One attractive approach is the selective targeting of MSC markers. Although the physiological function of prominin-1 in stem and CSC has not been elucidated, it is conceivable, based on data from our laboratory and others [6, 37], that prominin-1 represents a potential molecular target as well as cells expressing it. Efficacy of anti-prominin-1 immunotoxins was elegantly shown in hepatocellular and gastric cancer: the AC133 mAb, conjugated to monomethyl auristatin F, effectively inhibited the growth of Hep3B hepatocellular and KATO III

Table 13.1 Expression of prominin-1 in melanoma specimens

Reference	Specimen	Prominin-1 ⁺		Detection method	Comment
		cases/total			
Monzani, 2007 [5]	Distant metastasis	7/7		Flow cytometry	0.2% to 0.8% of cells positive
Klein, 2007 [3]	Primary melanoma	28/71		IHC	
	Distant metastasis	39/84			
Bongiorno, 2008 [27]	Lentigo maligna	Vast majority/30		IHC	Staining in the outer root sheath of mid-lower hair follicles
Piras, 2010 [28]	Primary melanoma	54/106		IHC	Staining at the invading front
	Nodal metastasis	17/32			
Gazzaniga, 2010 [29]	Sentinel lymph node	18/45		RT-PCR	
Fusi, 2011 [31]	Circulating melanoma cells	13/28		Flow cytometry	
	Distant metastasis	6/6		IHC	
Thill, 2011 [32]	Uveal melanoma	8/8		IHC	Staining at the invading front
Sharma, 2010 [34]	Primary melanoma	18/40		IHC	Statistically significant overexpression in lymph node and distant metastases compared to benign nevi
	Lymph node metastasis	12/15			
	Distant metastasis	13/19			

IHC immunohistochemistry, RT-PCR reverse transcriptase-polymerase chain reaction

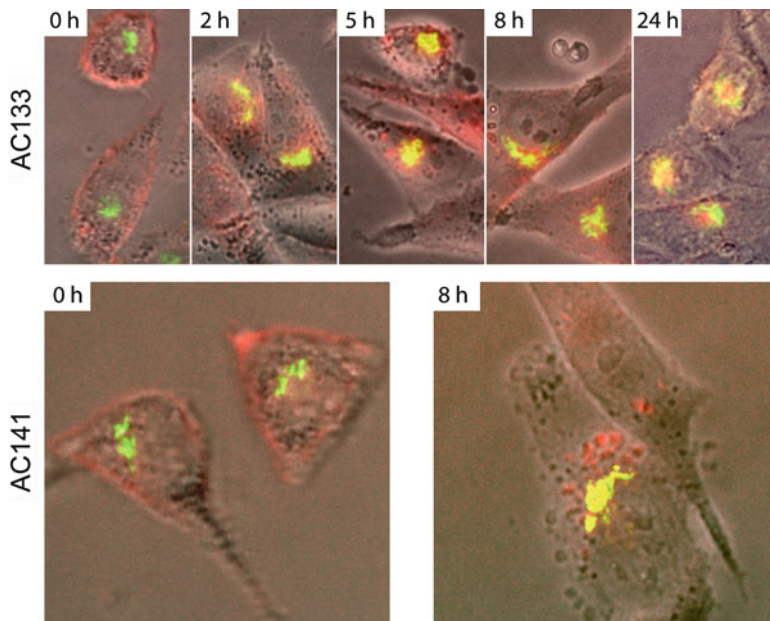


Fig. 13.1 Co-localization of anti-prominin-1-phycoerythrin (PE) antibodies with Golgi apparatus in human FEMX-I melanoma cells. The Golgi apparatus was labeled by a baculovirus expressing a signal peptide for N-acetylgalactosaminyltransferase fused to green fluorescent protein (GFP) under the control of a cytomegalovirus (CMV) promoter. *Left panel:* After 30 min, binding at 4°C with anti-prominin-1-PE antibody (AC133, *top panels*; AC141, *bottom panels*), cells were washed and incubated at 37°C for a given time (as indicated); *red*, anti-prominin-1-PE; *green*, GFP

gastric cancer cells *in vitro* and resulted in significant delay of Hep3B tumor growth in NOD-SCID mice [37].

Our laboratory performed preliminary studies to investigate the feasibility of an immunotoxin approach to malignant melanoma. First, we investigated whether anti-prominin-1 mAbs were internalized upon binding to prominin-1 on the surface of FEMX-I cells. To this aim, we incubated FEMX-I cells with phycoerythrin (PE)-conjugated anti-prominin-1 AC133 or AC141 mAbs for 30 min at 4°C (Fig. 13.1). The Golgi apparatus was highlighted by a baculovirus expressing a signal peptide for N-acetylgalactosaminyltransferase fused to green fluorescent protein (GFP) under the control of a cytomegalovirus (CMV) promoter. After extensive washing of the unbound antibody, cells were incubated at 37°C in an antibody-free medium. Progressively, the anti-prominin-1 antibodies were internalized and transported to the Golgi apparatus, with an apparent accumulation therein between 12 and 24 h.

We employed saporin, the ribosome-inactivating toxin derived from the seeds of *Saponaria officinalis*, for conjugation with the AC133 mAb, because of its previously reported efficacy in several xenograft models [38, 39], and it is simple to conjugate. The conjugated antibody had a 1.9 saporin/antibody molar ratio. For nontargeted saporin control, we used pre-immune mouse IgG antibody (with no known specificity) conjugated to saporin using the same protocol as the targeted immunotoxin.

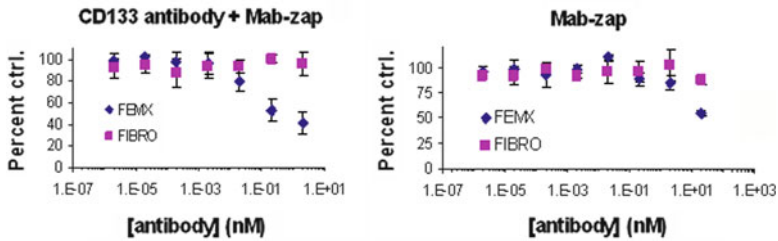


Fig. 13.2 In the presence of anti-prominin-1 monoclonal antibodies, saporin-conjugated secondary antibodies (Mab-zap) are toxic to prominin-1-expressing FEMX-I (FEMX) cells, but not to primary human fibroblasts (FIBRO)

To determine AC133/saporin conjugated as an immunotoxin, we evaluated by indirect and direct methods its cytotoxicity. For indirect immunotoxin assay, FEMX-I and normal adult human primary dermal fibroblasts (from the American Tissue Culture Collection) were plated at 1,000 per well (10,000/mL) onto 96-well plates in serum-supplemented tissue culture medium and incubated overnight at 37°C under 5% CO₂ atmosphere. 24 h later, cells were incubated with saporin-conjugated secondary antibody alone (Fig. 13.2, Mab-zap; right panel) or with AC133 mAb+Mab-zap (left panel). AC133 mAb and Mab-zap were mixed at 1:1 molar ratio (20 nM each) in serum-supplemented culture medium; as control, Mab-zap was incubated at the same concentration (20 nM) with serum-supplemented tissue culture medium. After 2–3 h at room temperature, mixing on a rotating platform, the antibody mixtures were added to the cells. Three days later, cytotoxicity was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay. We observed that, in the presence of anti-prominin-1 monoclonal antibody, saporin-conjugated secondary antibody was toxic to prominin-1-expressing FEMX-I cells, but not to control human fibroblasts (Fig. 13.2).

In a direct immunotoxin experiments, we compared the effects of saporin-conjugated AC133 mAb with saporin-conjugated pre-immune mouse IgG antibody on both FEMX-I and prominin-1-knockdown FEMX-I, the latter expressing only 15,000 prominin-1 molecules/cell. The saporin-conjugated AC133 antibody was much more effective on FEMX-I cells than on the prominin-1 knockdown, while the pre-immune conjugate showed modest toxicity only at 2-log higher concentrations (Fig. 13.3). These data indicate that, as expected, the level of expression of prominin-1 is an important determinant of the immunotoxin activity.

In summary, the rationale for development of anti-prominin-1 immunotoxins lies in (i) our previous findings that prominin-1 downregulation prevents the formation of metastasis [6]; (ii) our preliminary data showing that saporin-conjugated anti-prominin-1 antibodies are toxic to prominin-1-expressing FEMX-I cells, but not to control human fibroblasts, and that anti-prominin-1 antibodies are effectively internalized and localized not in the lysosomes, where immunotoxins and antibodies are rapidly degraded [40], but in the Golgi apparatus; and (iii) the recent report of *in vivo* antitumor efficacy of an anti-prominin-1-drug conjugate in prominin-1⁺ hepatocellular and gastric cancer cells *in vivo* [37]. However, before clinical translation,

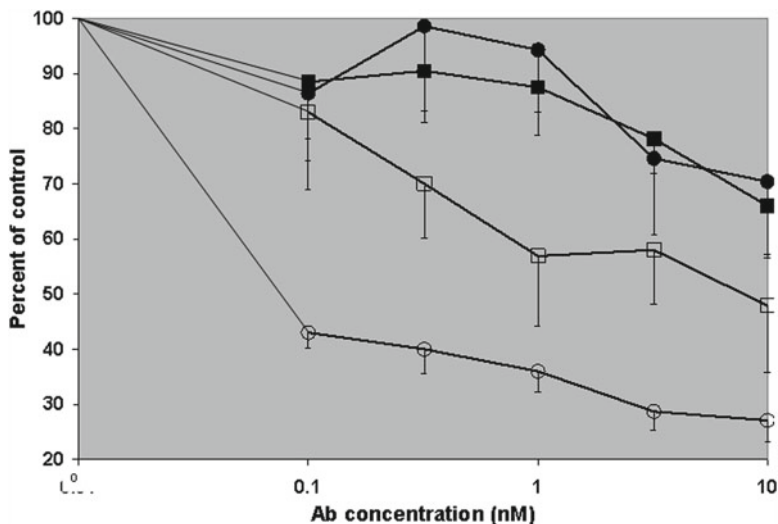


Fig. 13.3 Effects of saporin-conjugated AC133 antibody (*open symbols*) and pre-immune saporin conjugate (*solid symbols*) on FEMX-I (*circles*) and FEMX-I prominin-1-knockdown cells (*squares*). *Points* represent the mean of three separate experiments. *Bars*, standard deviation

the potential toxicity of prominin-1 targeting to nonmalignant prominin-1-expressing stem cell compartments should be investigated. The issue of potential toxicity to host tissues is crucial and difficult to tackle in mouse models because it is highly difficult to have a good handle on comparative toxicities. Furthermore, anti-prominin-1 immunotoxins could target and subsequently deplete a pool of long-term repopulating hematopoietic stem cells given the expression of prominin-1 in a subset of human CD34⁺ stem and progenitor cells [41–44]. The restricted localization of prominin-1 at the apical plasma membrane in polarized epithelial cells found in healthy human tissues [45] may limit however the accessibility of the antibody and thereby lower the risk of antigen-dependent toxicities. Often, cell polarization is lost, at least in poorly differentiated tumors, potentially enhancing the accessibility to antibody targeting [46].

Other prominin-1-based immunotherapeutic approaches have been proposed. In a paper by Pietra and colleagues [19], natural killer (NK) cells eradicated human melanoma cells with characteristics of CSC. While prominin-1⁺ melanoma cells preferentially survived radiations as compared with prominin-1⁻ cells, prominin-1⁺ melanoma cells displayed the same susceptibility to NK cell-mediated lysis than their prominin-1⁻ counterpart, thus suggesting that NK cell-based immunotherapy might be considered as a possible approach in the cure of patients with metastatic melanoma. Miyabayashi and colleagues [47] demonstrated that a prominin-1⁺ subpopulation in murine melanoma was immunogenic and that effector T cells specific for the prominin-1⁺ melanoma cells mediated potent antitumor reactivity, curing the mice of the parental melanoma. Prominin-1⁺ melanoma antigens preferentially induced type 17 T helper (Th17) cells and Th1 cells but not Th2 cells. Prominin-1⁺

melanoma cell-specific CD4⁺ T cell treatment eliminated not only prominin-1⁺ tumor cells but also negative ones while inducing long-lasting accumulation of lymphocytes and dendritic cells with upregulated MHC class II in tumor tissues. Furthermore, the treatment prevented the regulatory T cell induction [47]. The authors concluded that T cell immunotherapy is a promising treatment option to eradicate prominin-1⁺ drug-tolerant cells in cancerous tissues. It is of note that based on our recent finding of prominin-1-containing exosomes and on their potential involvement in the formation of melanoma metastases (see above), an alternative therapeutic approach could consist of drugs modulating either the exosome release or their uptake by supporting and/or neighboring cells. In line with this objective, recent data have suggested the endocytosis of hematopoietic cell-derived CD133-containing exosomes by microenvironmental cells [48].

Finally, our data describing a link between prominin-1 and Wnt pathway in FEMX-I cells suggest that key players of this signaling pathway might be potential targets to inhibit melanoma progression in prominin-1-expressing melanomas [6]. The relevance of Wnt signaling to the metastatic process has recently been unveiled by the observation that β -catenin is a central mediator of melanoma metastasis to the lymph nodes and lungs [23]. Prominin-1-mediated Wnt activation, which is probably linked to a dynamic organization of prominin-1-containing membrane microdomains [49], may be responsible for the precise coordination and integration of the expression of multiple genes, resulting in dynamic, pro-metastatic changes of cell adhesion and motility.

13.8 Conclusions

The refractoriness of metastatic melanoma to conventional therapy requires innovative approaches to prevention of metastatic dissemination or to specific targeting of the malignant population. Experimental data from our laboratory and others indicate a possible role of prominin-1 in the metastatic process. The frequent expression of prominin-1 in both melanoma cell lines and clinical melanoma specimens suggests that this molecule [and its underlying signaling pathway(s)] is a potential therapeutic target. A better understanding of the function of prominin-1 in melanoma cells as well as in healthy tissues and careful consideration of potential toxicities associated with its direct (or indirect) targeting is nevertheless required before clinical trials.

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