

## Melanoma Progression in a Changing Environment

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Melanoma research is considerably influenced by the implementation of novel techniques, e.g. array-based genomics and proteomics (see also Dr. Herlyn's commentary "Melanoma Research in a Changing Environment"), and has contributed to our understanding that tumor progression, including melanoma, is the result of the dynamic interaction between transformed cells and the microenvironment in which they thrive. Therefore, we have taken the liberty to adapt the title of Dr. Herlyn's commentary and briefly discuss the dynamic environment in which a melanoma develops and progresses.

### Introduction

The tumor stage of melanoma is mainly defined by two well-established parameters that are closely correlated with the patients' prognosis, i.e. thickness according to Breslow and microscopic ulceration. Earlier, Wallace H. Clark Jr. introduced levels of invasion and later discerned the relatively indolent, horizontal or radial growth phase (RGP) from the deeply invasive vertical growth phase (VGP) with metastasizing capacity. (See also Dr. Elder's commentary: "The Role of Histopathology in Melanoma Diagnosis and Prognosis"). During melanoma progression, changes in the expression of several molecules by neoplastic cells have been observed that correlate with melanoma progression. However, altered expression of relatively few molecular markers directly correlates with the transition from RGP to VGP melanoma. For instance, expression of the  $\alpha v \beta 3$  vitronectin receptor and ALCAM/CD166 emerges exclusively in VGP melanomas (Albelda et al., 1990; Hsu et al., 1998; van Kempen et al., 2000; Hsu et al., 2002; van Kempen et al., 2003). However, not all VGP melanomas express these molecules indicating that other yet unidentified mechanisms may exist that are involved in the transition to malignant melanoma growth. Recent work has shown that regulatory signals governing melanocytic cell growth and differentiation also originate from the surrounding host cells either directly through physical contact or indirectly through soluble factors and extracellular matrix molecules indicating that the tumor environment and

melanoma-stroma interactions can be critical determinants of neoplastic progression (Ruiter et al., 2002; van Kempen et al., 2003).

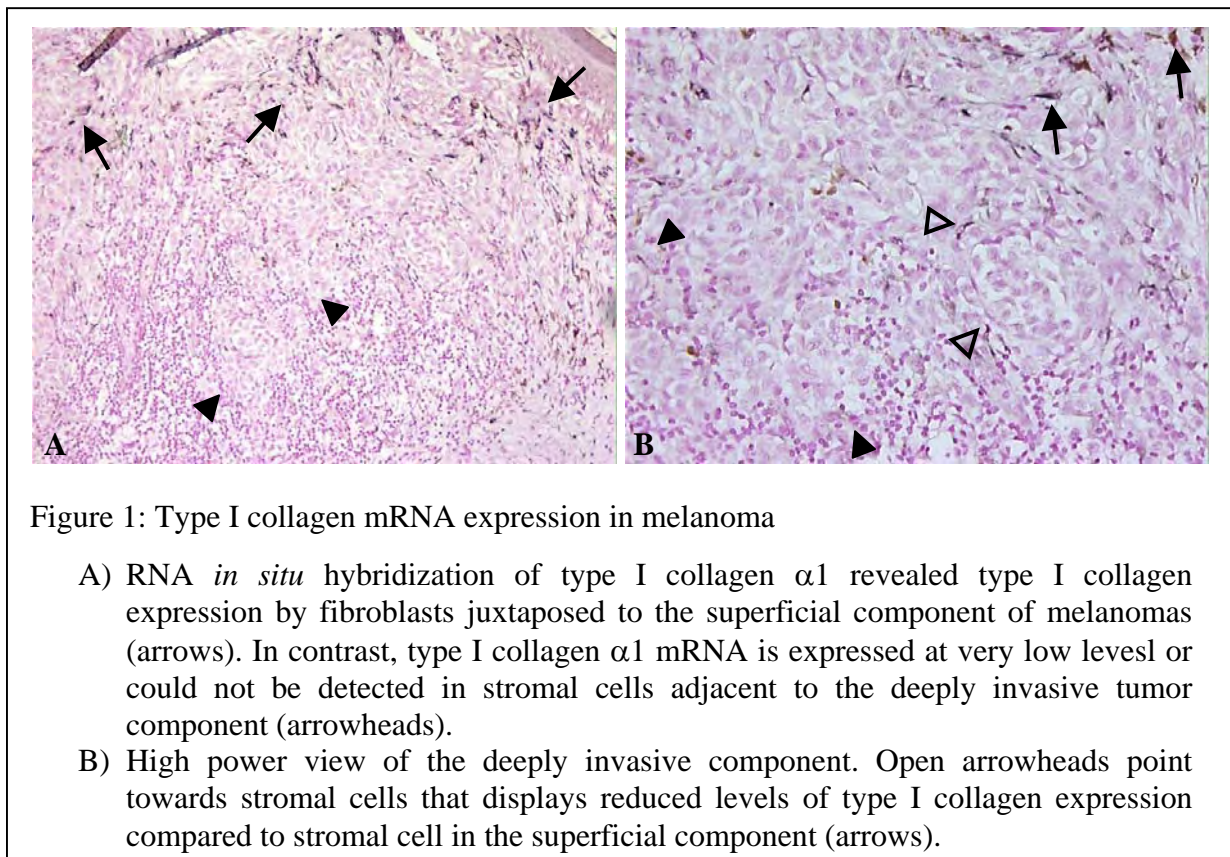
#### The tumor microenvironment facilitates angiogenesis

During early stages of melanoma progression, innate and adaptive immune cells are recruited towards the developing neoplasm. Especially macrophages have been implicated in the induction of angiogenesis by the release of granule-stored proteins that directly affect the local bioavailability of angiogenic molecules (e.g. basic fibroblast growth factor (bFGF/FGF2) and interleukin (IL)-8). In addition, the release of proteinases (e.g. matrix metalloproteinase (MMP)-9) can liberate extracellular matrix-sequestered angiogenic and mitogenic polypeptides. The inflammatory response can, thus, profoundly alter the local bioavailability of growth factors that can subsequently modulate melanoma development. In contrast to cutaneous squamous cell carcinomas, melanomas, but with the exception of the desmosplastic variants, do not elicit a strong stromal response with respect to fibroblast recruitment and myofibroblast differentiation, and deposition of extracellular matrix proteins (e.g. collagens). Fibroblasts are the major source of extracellular matrix proteins whose expression is also determined by environmental cues. Type I collagen synthesis by fibroblasts can be modulated by a variety of growth factors and cytokines that can be produced by melanoma and/or stromal cells (reviewed (van Kempen et al., 2003)). These data indicate that the local bioavailability of factors that can stimulate or inhibit collagen synthesis modulate collagen metabolism, and that activation of multiple signaling pathways is a prerequisite for collagen synthesis. Turnover of type I collagen is a critical determinant of angiogenesis. Collagen fibrils need to be degraded in order to allow sprouting vascular endothelial cells to elongate and form vascular tubes. The latter also requires synthesis of new collagen fibers that need to be aligned along the newly formed vessels. Thus, the coordinate action of inflammatory cells and fibroblasts induces and supports blood vessel formation. From this conclusion one can argue that ablation of the host response towards the developing melanoma and restoration of homeostasis of a non-tumor microenvironment could represent novel useful strategies for melanoma therapy.

#### Type I collagen expression and angiogenesis in primary melanoma

Angiogenesis is a hallmark of tumor progression, including melanoma. Nevertheless, anti-angiogenic agents have only been infrequently tested in patients with advanced melanoma. It therefore remains unclear if and why inhibition of angiogenesis in patients with VGP

melanoma could be an effective (adjuvant) therapy. We have demonstrated that vascular density correlates with melanoma thickness, *i.e.* increased angiogenesis but not lymphangiogenesis is observed in lesions thicker than 1.5 mm (De Waal et al., 1997; van Kempen et al., 2003). Importantly, a vascular network is formed adjacent to the upper component of the tumor, whereas the deeply invasive tumor component is relatively avascular. Because turnover of type I collagen synthesis can facilitate angiogenesis, we have recently analyzed its expression in melanoma (van Kempen et al., 2005). Interestingly, mRNA *in situ* hybridization revealed type I collagen expression by fibroblasts juxtaposed to melanoma cell nests within the upper part of the tumor (Figure 1).



In contrast, fibroblasts associated with deeply invasive nests of melanoma cells did not evidence type I collagen expression. This expression pattern correlates with the spatial organization of the vasculature and suggests that collagen expression during early melanoma development contributes to the formation of a melanoma-associated vasculature. Inhibition of collagen synthesis may therefore attenuate angiogenesis and inhibit further melanoma development. To test this possibility, we have employed the Melanoblastoma-bearing Libechov Minipigs (MeLiM) melanoma model. Similar to human melanomas, porcine melanomas develop from melanocytes within the epidermis. Melanoma development and

early progression closely resembles human melanoma progression with respect to skin architecture, epidermal localization of melanocytes, and histopathology of the tumors. MeLiM are born with or develop multiple highly pigmented melanocytic lesions within 6 weeks after birth and comprise intra-epidermal atypical melanocytic proliferations, superficial spreading and nodular melanoma (Vincent-Naulleau et al., 2004). Similar to human melanoma, MeLiM melanoma can be reported according to Clark levels. In our experiments piglets received halofuginone, an alkaloid that inhibits type I collagen expression, immediately after birth. We have observed that halofuginone attenuated collagen expression and the development of a tumor vasculature (van Kempen et al., manuscript submitted). Furthermore, it decreased post-natal melanoma incidence. Melanomas that did arise were not as deeply invasive as melanomas arising in control animals: a 60% decreased incidence of Clark level IV and V melanomas was observed. Importantly, we did not see an effect of the inhibition of collagen synthesis on the development and progression of melanocytic lesions that were already present at birth, indicating that inhibition of angiogenesis is not an effective treatment modality when the development of these lesions is already in progress. Collectively, these data demonstrate that the angiogenic switch is an early event during melanoma progression and that anti-angiogenic therapy of melanoma only exerts a positive effect when angiogenesis is inhibited at an early stage of the disease. Currently, the effects of oral administration of halofuginone as an anti-angiogenic drug (Tempostat – Collgard Biopharmaceuticals Ltd.) in solid tumors (including melanoma) is analyzed in a phase I/II clinical trial, which is orchestrated by the European Organisation for Research and Treatment of Cancer. Our recent results would predict that halofuginone may not be an effective therapeutic strategy for melanoma when collagen synthesis is not inhibited during early stages of tumor development. Otherwise, halofuginone could add up to the list of potential drugs that gave disappointing results in the clinical trials (e.g. angiogenesis inhibitors and inhibitors of matrix metalloproteinases) when it is not realized at what stage of tumor progression collagen turnover and angiogenesis actually contribute to tumor progression.

To our opinion, if a melanocytic lesion with increased risk of malignancy cannot be excised readily (e.g. lentigo maligna of the face) halofuginone and other anti-angiogenic compounds are more suitable as chemoprevention drugs to attenuate the transition of micro-invasive to overtly invasive melanoma.

### Anti-angiogenic therapy of metastatic melanoma

Because primary melanomas are most often easily removed surgically, anti-angiogenic therapy will not be a first line treatment modality for melanoma anyway. Can these therapies be of clinical value for treatment of metastatic melanoma? Clinical trials with a large variety of inhibitors have revealed that blocking blood vessel growth is often not the magic bullet that we have been hoping for: whereas tumor angiogenesis can be blocked, it often does not result in complete reduction of tumor burden. Preclinical studies predominantly comprise subcutaneous xenografts models, in which tumor growth is dependent on angiogenesis. Evidence is accumulating that when primary tumors or metastases in highly vascularized tissue are challenged with anti-angiogenic agents, it can co-opt pre-existing vessels (reviewed in (van Kempen and Leenders, 2006)). Especially for melanoma metastases growing in highly vascularized organs (e.g. liver, lung and brain), inhibition of angiogenesis can induce a tumor to adapt to a vessel co-optive phenotype that results in tumor growth in the perivascular space of pre-existing vessels. In addition, inhibition of angiogenesis most likely does not affect vasculogenic mimicry by melanoma cells. In summary, although clinical data of anti-angiogenic therapy of metastatic melanoma is not available, we predict that melanoma patients will not benefit from this type of treatment.

### Does the stroma of advanced primary and metastatic melanoma provide targets for therapy?

Reporting the level of melanoma invasion according to Clark levels is less reproducible than measurement of thickness according to Breslow (McDermott et al., 1998). This is also applicable to the estimation of the growth phase, although in experienced hands it has good agreement. Apart from the assessment of prognosis, the Clark levels are highly informative on the environment in which the tumor is growing. Tumors confined to the papillary dermis (Clark II-III) are embedded within a finely woven meshwork of type I collagen fibers. In contrast, melanoma cells within the reticular dermis (Clark IV) encounter a distinctive pattern of thick type I collagen bundles. The environmental cues originating from these collagen matrices remain largely elusive, and very little is known about the subcutaneous fat (Clark V). Importantly, inflammatory cells are found in close proximity of the relatively superficial component of the tumor (Clark I- III), but not with the deeply invasive component in the reticular dermis or subcutaneous fat. In addition, type I collagen synthesis by fibroblast and the angiogenic response primarily occur within the papillary dermis (Clark I-III) and not within the underlying tissue. It thus appears that deeply invasive melanoma cells have acquired a phenotype that is independent of stromagenesis, including angiogenesis.

Furthermore, these melanoma cells apparently invade tissue without profoundly changing the environment in which they grow. This is in strong contrast to other tumors, e.g. cutaneous squamous cell carcinoma, where tumor cell invasion does not remain unnoticed by the host cells. Growth of these tumors appears to depend on the stromal response and this tumor microenvironment may thus contain targets for anti-tumor therapy. In contrast, the environment of deeply invasive melanoma may thus not contain stromal targets for therapy because it appears more or less unaffected by the tumor cells.

### Concluding remarks

With increasing tumor thickness not only quantitative changes, but also qualitative changes regarding both melanoma cells and the microenvironment occur. Tumor thickness, however, is a better prognostic factor than level of invasion: e.g. in spite of its superficial localization, thick (>1.7 mm) cutaneous polypoidal melanomas predict poor disease outcome (Reed et al., 1986). In these tumors, the increase in volume encourages dislodgement of melanoma cells that are carried to superficial lymphatic vessels, resulting in a poor prognosis (Plotnick et al., 1990). But the majority of the cutaneous melanomas display invasion of the dermis. We hypothesize that the stromal response predominantly occurring in the papillary dermis (e.g. inflammation, extracellular matrix remodeling, fibroblast activation) exerts a selective pressure which gives rise to metastasis competent melanoma cells that deeply invade the dermis without causing a stromal response. Preventing and reverting this “stealth phenotype” is one of the challenges for future melanoma research. An important issue that needs to be addressed is why deeply invasive melanoma cells seem unnoticed. Do melanoma cells adopt a gene expression program that leaves them undetected by host cells, including immune competent cells (e.g. by loss of HLA antigens)? Alternatively, do these invasive melanoma cells arise after the fusion of transformed melanocytes with macrophages? (See Dr. Pawalek’s contribution to this forum: “Viewing malignant melanoma cells as macrophage-tumor hybrids”.) By the acquisition of a macrophage phenotype, this would simultaneously increase the migratory potential of the tumor cells and would result in a wolf in sheep’s clothing. Thus, can the poor results of the allogeneic and autologous vaccine strategies in melanoma (see Dr. Sondak’s contribution: “Melanoma Vaccines: What have we learned?”) also be explained by the possibility that the melanoma cells are just not recognized by the immune system?

Although many questions still need to be answered, we are convinced that future research will yield an effective anti-melanoma therapy. Although its incidence is low, spontaneous melanoma regression can occur. Therefore a mechanism must exist to revert melanoma

growth. The latter is also observed in the porcine melanoma model that we have used (Vincent-Naulleau et al., 2004). In contrast to human melanoma however, all melanomas and their metastasis (!) disappear. The regression phase starts approximately 50-60 days after birth and culminates in a total depigmentation of the animal. Therefore, this model may provide important clues that can result in an effective melanoma treatment strategy.

We highly appreciate critical discussion of this commentary.

#### Reference List

Albelda,S.M., Mette,S.A., Elder,D.E., Stewart,R., Damjanovich,L., Herlyn,M., and Buck,C.A. (1990). Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. *Cancer Res.* *50*, 6757-6764.

De Waal,R.M., van Altena,M.C., Erhard,H., Weidle,U.H., Nooijen,P.T., and Ruiter,D.J. (1997). Lack of lymphangiogenesis in human primary cutaneous melanoma. Consequences for the mechanism of lymphatic dissemination. *Am. J. Pathol.* *150*, 1951-1957.

Hsu,M.Y., Meier,F., and Herlyn,M. (2002). Melanoma development and progression: a conspiracy between tumor and host. *Differentiation* *70*, 522-536.

Hsu,M.Y., Shih,D.T., Meier,F.E., Van Belle,P., Hsu,J.Y., Elder,D.E., Buck,C.A., and Herlyn,M. (1998). Adenoviral gene transfer of beta3 integrin subunit induces conversion from radial to vertical growth phase in primary human melanoma. *Am. J. Pathol.* *153*, 1435-1442.

McDermott,N.C., Hayes,D.P., al-Sader,M.H., Hogan,J.M., Walsh,C.B., Kay,E.W., and Leader,M.B. (1998). Identification of vertical growth phase in malignant melanoma. A study of interobserver agreement. *Am. J. Clin. Pathol.* *110*, 753-757.

Plotnick,H., Rachmaninoff,N., and VandenBerg,H.J., Jr. (1990). Polypoid melanoma: a virulent variant of nodular melanoma. Report of three cases and literature review. *J. Am. Acad. Dermatol.* *23*, 880-884.

Reed,K.M., Bronstein,B.R., Mihm,M.C., Jr., and Sober,A.J. (1986). Prognosis for polypoidal melanoma is determined by primary tumor thickness. *Cancer* *57*, 1201-1203.

Ruiter,D., Bogenrieder,T., Elder,D., and Herlyn,M. (2002). Melanoma-stroma interactions: structural and functional aspects. *Lancet Oncol.* *3*, 35-43.

van Kempen,L.C. and Leenders,W.P. (2006). Tumours can adapt to anti-angiogenic therapy depending on the stromal context: Lessons from endothelial cell biology. *Eur. J. Cell Biol.* *85*, 61-68.

van Kempen,L.C., Ruiter,D., van Muijen,G.N., and Coussens,L.M. (2003). The tumor microenvironment: a critical determinant of neoplastic evolution. *Eur. J. Cell. Biol.* *82*, 539-548.

van Kempen,L.C., van den Oord,J.J., van Muijen,G.N., Weidle,U.H., Bloemers,H.P., and Swart,G.W. (2000). Activated leukocyte cell adhesion molecule/CD166, a marker of tumor progression in primary malignant melanoma of the skin. *Am. J. Pathol.* *156*, 769-774.

van Kempen,L.C., van Muijen,G.N., and Ruiter,D.J. (2005). Stromal responses in human primary melanoma of the skin. *Front Biosci.* *10*, 2922-2931.

Vincent-Naulleau,S., Le Chalony,C., Leplat,J.J., Bouet,S., Bailly,C., Spatz,A., Vielh,P., Avril,M.F., Tricaud,Y., Gruand,J., Horak,V., Frelat,G., and Geffrotin,C. (2004). Clinical and histopathological characterization of cutaneous melanomas in the melanoblastoma-bearing Libechev minipig model. *Pigment Cell Res.* *17*, 24-35.