

# Matricellular Proteins Produced by Melanocytes and Melanomas: In Search for Functions

Mizuho Fukunaga-Kalabis · Ademi Santiago-Walker · Meenhard Herlyn

Received: 4 January 2008 / Accepted: 28 February 2008 / Published online: 26 March 2008  
© The Author(s) 2008

**Abstract** Matricellular proteins are modulators of cell-matrix interactions and cellular functions. The group includes thrombospondin, osteopontin, osteonectin/SPARC, tenascin, disintegrins, galectins and CCN proteins. The production of matricellular proteins such as osteopontin, SPARC or tenascin is highly upregulated in melanoma and other tumors but little is known about their functions in tumor growth, survival, and metastasis. The distribution pattern of CCN3 differs from most other matricellular proteins, such that it is produced abundantly by normal melanocytes, but is not significantly expressed in melanoma cells. CCN3 is known to inhibit melanocyte proliferation and stimulate adhesion to collagen type IV, the main component of the basement membrane. CCN3 has a unique role in securing adhesion of melanocytes to the basement membrane distinct from other melanoma-produced matricellular proteins which act as de-adhesive molecules and antagonists of focal adhesion. Qualitative and quantitative changes in matricellular protein expression contribute to melanoma progression similar to the E-cadherin to N-cadherin class switch, allowing melanoma cells to escape from keratinocyte control.

**Keywords** Melanoma · Matricellular proteins · CCN3 · SPARC · Tenascin C · Osteopontin

## Abbreviations

CCN cystein rich 61, connective tissue growth factor, nephroblastoma overexpressed

SPARC	secreted protein, acidic and rich in cysteine
MMP	matrix metalloproteinase
IL-1 $\beta$	interleukin-1beta
Cx43	connexin 43
UV	ultra violet
NF- $\kappa$ B	nuclear factor kappa B
TGF- $\beta$	transforming growth factor beta
CSC	cancer stem cell

## Introduction

During development, melanocyte precursors migrate from the neural crest toward the epidermis, where they are arrested upon contact with keratinocytes. Differentiated human melanocytes are specifically localized to the basement membrane and cannot survive within the upper epidermal layers unless transformed, forming nevi or melanomas. In turn, melanocyte homeostasis is strictly controlled by the microenvironment. Dysregulation of homeostasis disturbs the balance of the epidermal melanin unit and may trigger the continuous proliferation of melanocytes, leading to the development of pigmented lesions. It is likely that melanoma cells escape from physiological control through: (1) down-regulation of receptors important for their communication with and adhesion to keratinocytes (e.g. E-cadherin); (2) up-regulation of receptors and signaling molecules not found on melanocytes but important for melanoma–melanoma, melanoma–fibroblast, and melanoma endothelial cell interactions [e.g. N-cadherin, melanoma cell adhesion molecule (Mel-CAM), zonula occludens protein-1 (ZO-1)]; and (3) loss of anchorage to the basement membrane due to altered expression of extracellular-matrix (ECM) binding proteins [1].

M. Fukunaga-Kalabis · A. Santiago-Walker · M. Herlyn (✉)  
Molecular and Cellular Oncogenesis Program,  
The Wistar Institute,  
3601 Spruce Street,  
Philadelphia, PA 19104, USA  
e-mail: herlynm@wistar.org

Of ECM proteins, matricellular proteins are a sub-class first proposed by Bornstein [2]. The term ‘matricellular’ has been applied to a group of extracellular proteins that do not contribute directly to the formation of structural elements in vertebrates but serve to modulate cell–matrix interactions and cellular functions [3]. Depending on cell type and tissue context, matricellular proteins participate in diverse processes, such as cell adhesion, proliferation, differentiation, and survival [4, 5].

The original group of matricellular proteins was composed of thrombospondin-1 (TSP1), SPARC (secreted protein, acidic and rich in cysteine; also known as osteonectin), and tenascin C, and was more recently expanded by the inclusion of TSP2, osteopontin, tenascin X, disintegrins, galectins, and CCN proteins. The production of matricellular proteins such as osteopontin, SPARC or tenascin is highly upregulated in melanoma and other tumors, but little is known about their functions in tumor growth, survival, and metastasis. Recently we reported that CCN3 (NOV, nephroblastoma overexpressed) is upregulated in melanocytes after co-culture with keratinocytes and that it affects two fundamental features of melanocyte physiology: it inhibits the proliferation of melanocytes, and is required for the proper localization of the melanocyte network on the basement membrane of human skin [6].

This review will first focus on the putative role of CCN3 in melanocyte physiology, and why dysregulation of its expression may play a role in the onset of melanoma. The molecular mechanisms underlying dysregulation of CCN3 will also be discussed. In addition, recent work on other matricellular proteins in melanoma will be summarized. Finally, we will examine novel evidence indicating a role for matricellular proteins as regulators of the niche in diverse stem cell systems including tumor stem cells.

### CCN3 Function in Melanocytic Cells

#### CCN3: Contribution to Melanocyte Physiology

In the normal human epidermis, the phenotype of melanocytes is delicately regulated by the epidermal micro-environment. Keratinocytes control the proliferation of melanocytes in order to maintain a lifelong stable keratinocyte to melanocyte ratio. Epithelial keratinocytes also regulate the expression of cell surface molecules on melanocytes [7, 8]. Keratinocytes produce growth factors and cytokines that act as paracrine factors in regulating the phenotype of melanocytes, including: interleukin-1beta (IL-1 $\beta$ ), tumor necrosis factor-alpha (TNF- $\alpha$ ), stem cell factor (SCF), epidermal growth factor (EGF), and endothelin 1 [9–11].

In a search for molecular players involved in the crosstalk between human melanocytes and keratinocytes, CCN3 was found to be upregulated in melanocytes after co-culture with keratinocytes [6]. CCN3 belongs to the CCN protein family which consists of six members including Cyr61 (cystein rich 61; CCN1), CTGF (connective tissue growth factor; CCN2), NOV (nephroblastoma overexpressed gene; CCN3), WISP-1, 2 and 3 (Wnt-1 induced secreted proteins; CCN4–6) [12]. CCN proteins contain structural motifs including insulin-like growth factor binding protein-like domains, von Willebrand factor type C repeats, thrombospondin type 1 repeats, and C-terminal cysteine knot modules [12]. CCN proteins are involved in the regulation of cell proliferation, migration, attachment, and differentiation. During embryonic development, CCN3 expression has been widely observed in derivatives of all three germ layers, specifically in skeletal muscle, smooth muscle of vessel walls, nervous system, adrenal cortex, and differentiating chondrocytes [13]. In human skin, CCN3 is expressed in the basal layer of the epidermis, where melanocytes are positioned [6]. Keratinocytes in culture do not express CCN3, whereas melanocytes constitutively express it at low levels. When melanocytes are co-cultured with keratinocytes, CCN3 is strongly expressed in the cytoplasm of melanocytes and secreted into the culture medium. Keratinocyte-derived pro-inflammatory cytokines such as IL-1 $\beta$  or TNF- $\alpha$  stimulate CCN3 expression and secretion.

Abrogation of CCN3 in melanocytes leads to aberrant phenotypes in human organotypic skin cultures, exhibiting melanocyte hyper-proliferation and disorganization of the three-dimensional melanocyte network on the basement membrane. These findings support the importance of keratinocyte–melanocyte crosstalk in the control of melanocyte phenotype in human skin [1].

CCN3 is the prototype of anti-proliferative CCN proteins [14]. It regulates proliferation in both benign and malignant cells including fibroblasts, glomerular cells, glioblastoma, Ewing’s sarcoma cells, and chronic myeloid leukemia cells [15–17]. However, the exact mechanisms responsible for growth inhibition remain to be explored. Bleau et al. [14] showed that production of CCN3 varies throughout the cell cycle and it accumulates at the G2/M transition. Reportedly, the CT module of CCN3 is sufficient to induce cell growth inhibition. This module physically interacts with Connexin 43 (Cx43) [16, 18]. In rat glioblastoma cells, CCN3 co-localizes with Cx43 in plaques at the plasma membrane, suggesting that an interaction of CCN3 with the C terminus of Cx43 could play an important role in mediating growth control by specific gap junction proteins [16]. In human skin, Cx43 is abundantly expressed in the suprabasal layers [19]. During pathological changes leading to melanoma development, in addition to the cadherin class switch,

changes in connexin expression, in particular loss of Cx43, results in a reduction or loss of gap junctional activity thought to contribute to tumor progression [1]. This is consistent with the observation that CCN3 is expressed at the dermo-epidermal junction in human skin where melanocytes and keratinocytes are in close contact [6].

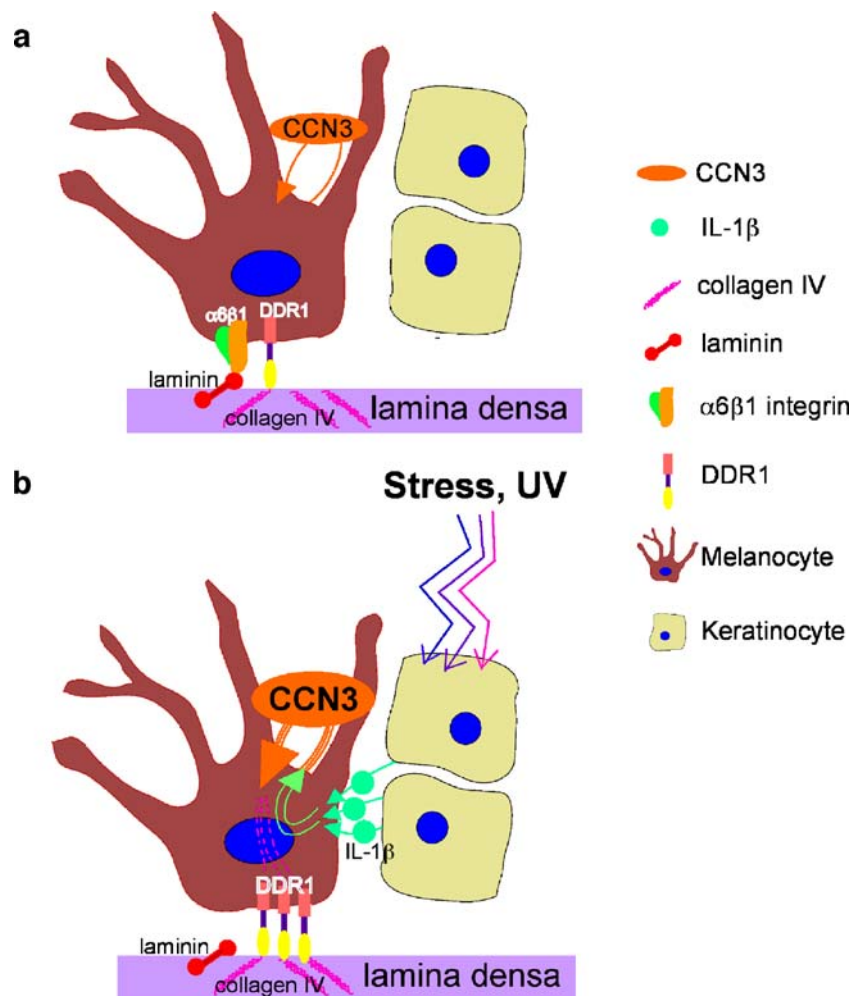
Melanocytes appear to have a contingency mechanism essential for their survival that secures continuous attachment to the basement membrane of the skin. The primary mechanism for attachment is reportedly through integrin(s) [20], of which the laminin-binding integrin  $\alpha 6\beta 1$  is the main candidate [21, 22]. Since expression of the  $\alpha 6$  integrin subunit is downregulated by ultraviolet irradiation [23], melanocytes must develop alternative mechanisms to maintain localization at the basement membrane. Interestingly, CCN3 production by melanocytes, in response to keratinocyte contact, has important consequences for melanocyte adhesion to basement membrane collagen IV [6]. Upon CCN3 stimulation melanocytes increase expression of discoidin domain receptor 1 (DDR1), a receptor

tyrosine kinase and a receptor for collagen [24]. Thus DDR1, as a collagen IV receptor, prevents melanocytes from separating from the basement membrane, a critical safety mechanism during inflammatory skin reactions in preventing apoptosis (Fig. 1).

It is not clear yet how CCN3 signals to induce DDR1 upregulation. DDR1 is a direct p53 transcriptional target [25]. Another CCN protein, CCN1 can activate the beta-catenin/TCF4 complex, which promotes the expression of c-myc followed by activation of p53 [26]. It is possible that CCN3 upregulates DDR1 expression through activation of p53 since p21, one of downstream targets of p53, is upregulated in CCN3-treated cells [6].

CCN3 binds to cell surface integrins and induces intracellular signaling [5]. Neither overexpression nor abrogation of CCN3 in melanocytes affect their adhesion to laminin, the main ligand for  $\alpha 6$  integrin, suggesting that CCN3- $\alpha 6$  integrin binding is not essential for anchorage of melanocytes to the basement membrane. CCN3 can bind to  $\alpha \nu \beta 3$  [27], a multi-ligand binding integrin, however the  $\beta 3$

**Fig. 1** Proposed role of CCN3 in melanocyte adhesion. **a** In resting skin, melanocytes are localized to the basement membrane via multiple adhesion mechanisms including integrin-laminin binding and/or DDR1-collagen IV binding. **b** Under conditions of stress such as ultra violet (UV) irradiation, integrin-laminin connections are disrupted. A potential mechanism by which melanocytes maintain adhesion to the basement membrane involves the production and release of IL-1 $\beta$  by keratinocytes in response to stress. IL-1 $\beta$  stimulates CCN3 production and secretion by melanocytes. CCN3 then acts in an autocrine manner, to enhance collagen receptor DDR1 expression in melanocytes, promoting adhesion to collagen IV in the basement membrane



subunit is not expressed by normal melanocytes [21, 28]. CCN3 can also bind Notch [29]; though Notch signaling is not activated in melanocytes by CCN3 overexpression (unpublished data). In summary, growth inhibition and basement membrane localization conferred by CCN3 are important, if not essential, functions for maintaining melanocyte homeostasis in normal skin.

### CCN3: Altered Expression in Melanoma

CCN3 is aberrantly overexpressed in several malignancies and is associated with the progression of prostate cancer [30], renal cell carcinoma [31] and Ewing's sarcoma [32], whereas in rhabdomyosarcoma and cartilage tumors, increased CCN3 expression correlates with tumor differentiation. Extensive studies have indicated that the biological properties of CCN3 are dependent upon the cellular context [33]. Thus, it is not surprising that CCN3 has diverse effects on tumorigenesis in different types of cancer. For example, CCN3 has antiproliferative activities in human and rat glioblastomas [34] and human chronic myeloid leukemias [35], whereas it promotes migration and invasion of human Ewing's sarcoma cells [17] as well as adhesion and migration in human glioblastoma cells [36].

CCN3 was characterized as the first member of the matricellular protein family that is downregulated in aggressive human melanomas [37]. The pathological progression of melanoma can be defined in five distinct stages [38]. The first stage is characterized by hyperplasia of melanocytes as seen in common acquired and congenital nevi. Dysplastic nevi with cytological and architectural atypia define the second stage. The third stage is radial growth phase (RGP) melanoma in which tumor cells are present within the epidermis or individually invade into the superficial dermis, but show little capacity to leave the primary site. In the fourth stage, the vertical growth phase (VGP), a population of melanoma cells invades deep into the dermis and subcutaneous tissue as an expanding cluster, increasing the risk for systemic dissemination. Finally, metastasis is the most advanced stage of melanoma. Taken together, the processes leading to the development of melanoma can be described as a disruption of homeostatic mechanisms in the skin. Such mechanisms control when and how cells proliferate, differentiate and undergo apoptosis in the epidermal melanin unit [39]. The disruption of homeostatic controls can lead to the progression of melanoma where cell–cell and cell–matrix crosstalk play key roles.

Immunohistochemical staining of melanoma lesions revealed that CCN3 expression is inversely correlated with tumor thickness. In contrast, other major proteins in the same family, such as osteopontin [40], tenascin C [41] or SPARC [42], are strongly upregulated in human melanoma

cells compared with normal melanocytes. These data suggest that qualitative and quantitative shifts of matricellular proteins contribute to melanoma progression similar to the cadherin class switch. The cadherin switch is characterized by the downregulation of E-cadherin in melanoma cells when compared to melanocytes and by the upregulation of N-cadherin, allowing melanoma cells to escape from keratinocyte control [43]. The switch in matricellular proteins suggests a potential role for CCN3 as an antagonist to melanoma-associated matricellular proteins such as osteopontin or tenascin C. In addition, the lack of CCN3 expression in advanced melanomas correlates well with Breslow's depth of invasion, one of the most important prognostic markers in melanoma, suggesting that CCN3 expression could be a potential marker for good prognosis.

Re-expression of CCN3 in an advanced human melanoma cell line decreased melanoma invasion through Matrigel by inhibiting matrix metalloproteinase (MMP) expression [37]. Several reports suggest that MMP-2/-9 activity or expression is regulated via the MAP kinase pathway [44–46]. Therefore, it is possible that CCN3 downregulates MMP-2/-9 through the inhibition of ERK. It has been suggested that after secretion of full-length CCN3 and cleavage of the extracellular compartment, the C-terminal portion of the protein could re-enter the cell and be routed to the nucleus via a nuclear localization signal [47]. Nuclear CCN proteins may, in complex with other regulatory proteins, act as transcriptional corepressors [47]. Therefore it is conceivable that CCN3 might negatively regulate MMP-2/-9 transcription. The activity of the CCN3 molecule appears to occur in the basement membrane zone, a location enriched with collagen IV which is a substrate of MMP-2/-9. Whether CCN3 prevents invasion by inhibiting the collagenase activity of MMP-2/-9 has yet to be determined. The use of skin reconstruct xenografts on nude mice might help determine whether CCN3 prevents cancer cells from escaping the ECM and initiating tumor invasion.

These results, together with those from melanocyte studies indicate that CCN3 has an inhibitory effect on human melanoma progression at least in the early stages. However, more studies are required before concluding that CCN3 acts as a tumor suppressor gene in melanoma. Although CCN3 suppressed the phosphorylation of ERK and the proliferation of melanoma cells *in vitro*, a significant reduction in tumor growth in a subcutaneous xenograft model was not observed (unpublished data). This finding contrasts with previous studies reporting an antiproliferative role for CCN3 *in vivo* [17, 34]. While CCN3 expression is decreased or lost in most lymphnode and cutaneous melanoma metastases [37], CCN3 expression is detected in visceral metastases [48]. Vallacchi et al. reported that induction of CCN3 in human melanoma cells increased adhesion to collagen I, vitronectin and laminin in

vitro as well as enhanced metastatic potential to specific organs such as liver and adrenal cortex *in vivo*. Their findings suggest that if melanoma cells spreading from the primary tumor maintain expression of CCN3, they tend to metastasize to visceral organs rather than lymphnodes or skin. These differing outcomes might reflect variability in molecular mechanisms of CCN3 signaling in different cellular contexts. Because normal melanocyte growth and adhesion are regulated by CCN3 [6], the biological functions of CCN3 in melanoma may depend, in part, on the cellular context at a given stage of tumor progression. CCN3 induces neovascularization when expressed in rat cornea [27], suggesting that it promotes angiogenesis. Because there is considerable heterogeneity among vascular endothelial cells, it is not clear whether CCN3 would also induce angiogenesis in a malignant tumor. This remains to be investigated.

### Matricellular Protein Switch in Melanoma Progression

Cancers are known as wounds that do not heal [49, 50]. During cancer development, tumor cells often utilize mechanisms of embryonic development and tissue repair regulated by interactions between cancer cells, activated stromal cells, and components of the extracellular matrix including matricellular proteins. Matricellular proteins are expressed primarily during development and growth, and in response to injury. They are abundant in tissues with continued turnover, such as bone [51, 52]. SPARC, tenascin C and osteopontin are matricellular proteins highly expressed in melanomas and a wide range of human malignant neoplasms.

#### SPARC in Melanoma

SPARC, also known as osteonectin, is a 43-kDa ECM glycoprotein involved in cell–ECM interactions during wound healing, tissue remodeling, and cancer progression [51]. Clinically, SPARC expression correlates with aggressiveness of melanomas and the acquisition of metastatic phenotypes [42]. Ectopic expression of  $\beta 3$  integrin, a key marker of VGP/metastatic melanoma, induces SPARC expression in human RGP melanoma cells [53]. SPARC has three general functions: de-adhesion, antiproliferation, and regulation of extracellular matrix (ECM) interactions [54]. SPARC suppresses expression of E-cadherin through up-regulation of Snail, leading to migratory and invasive behavior [55, 56]. Data strongly suggest that SPARC induces epithelial–mesenchymal transition and contributes to transformation of melanocytes. Recently a high-throughput study using cDNA microarrays revealed that expression of EMT-related genes including SPARC/osteonectin is

significantly associated with melanoma metastasis [57]. In addition to downregulation of E-cadherin, SPARC over-expressing melanoma cells showed upregulation of osteopontin and increased phosphorylation of focal adhesion kinase (FAK), suggesting that SPARC interacts with integrin-linked kinase (ILK) at focal adhesions to modulate cell–ECM interactions and promote an invasive melanoma phenotype [56]. SPARC produced by human melanoma cells also regulates inflammatory processes to inhibit polymorphonuclear (PMN) leukocyte recruitment and anti-tumor cytotoxic activity [58], suggesting that SPARC contributes to the innate immune response in cancer thereby promoting melanoma cell survival.

Since SPARC is not only expressed by tumor cells, but also secreted by surrounding fibroblasts and endothelial cells, it is likely that SPARC produced by tumor-infiltrating stromal cells plays a role in tumor progression. Prada et al. [59] reported that the growth capacity of human melanoma cells depends on SPARC levels produced by melanoma cells rather than stromal cell-derived SPARC. It is not clear yet why SPARC does not act in a paracrine manner in melanoma. It has been reported that SPARC functions are regulated by proteolytic cleavage [60, 61]. Furthermore, SPARC is observed both in the cytoplasm and in culture medium; thus, SPARC functions not only as an extracellular protein but likely also functions intracellularly [62]. It is conceivable that the location and concentration of SPARC, whether it is intact or proteolyzed, and interactions between SPARC and other molecules contribute to the impact of SPARC on target cells [62]. Several studies have confirmed that SPARC promotes tumor progression in glioma [63], while it reduces tumor activities in breast cancer [64]. These inconsistent observations suggest that regulation and function of SPARC are dependent on cellular context.

#### Tenascin C in Melanoma

Tenascin C is a large, 220–320 kDa per monomer, glycoprotein of the extracellular matrix secreted from cells as a hexamer of six identical chains, termed a hexabrachion [65]. Tenascin C has an N-terminal oligomerization motif, and EGF-like, fibronectin type III and fibrinogen-like domains. For most cells, tenascin C acts as an anti-adhesive molecule. When presented as soluble protein to cells in a strong adhesive state, Tenascin C acts by inducing a rapid transition to an intermediate state of adhesiveness characterized by loss of actin-containing stress fibers and restructuring of the focal adhesion plaque including loss of vinculin and alpha-actinin, but not of talin or integrin [66]. Tenascin C inhibits fibronectin-dependent adhesion [67]. These results indicate that tenascin C may play an important role in cell–matrix interactions. Although tenascin C binds multiple integrins, it is not clear whether these

interactions account for the many effects attributed to tenascin C [65].

Most human melanoma cells secrete tenascin C *in vitro* constitutively [41]. Transforming growth factor beta 1 (TGF- $\beta$ 1) increased secretion in tenascin-producing cells. Tenascin C was present in sera of melanoma patients, with significantly elevated levels in patients with advanced melanomas as compared to patients with low tumor burden or normal donors. Tenascin C expression is moderately increased in benign and dysplastic melanocytic tumors, greatly increased in melanomas and further increased in metastases [68]. Expression in invasive and metastatic melanomas is highest at the invasive fronts. The intensity of tenascin C staining correlates with metastasis to sentinel lymph nodes more consistently than tumor thickness [69, 70]. The main microenvironmental changes underlying metastasis include clustered migration of cancer cells, ECM degradation, paracrine loops of released growth factors and/or induction of adhesion molecules in stromal cells [71]. Adhesion regulated by tenascin C contributes to cancer progression by facilitating cell migration and reducing cell death from anoikis [66]. Tenascin C is also involved in the regulation of MMPs contributing to ECM degradation. It stimulates glioma cell invasion through MMP-12 activation [72]. Co-stimulation of human breast cancer cells with transforming growth factor-beta and tenascin C enhances MMP-9 expression and cancer cell invasion [73]. The large splice variant of Tenascin C (320 kDa) stimulates MMP-1 expression [74]. The molecular mechanisms underlying tenascin C-induced MMP activation remain to be elucidated.

Stromal cells also produce tenascin C. Myofibroblasts appear to be modified fibroblasts which express alpha-smooth muscle actin, the actin isoform typical of vascular smooth muscle cells, and they actively synthesize robust amounts of collagen and other ECM components [75]. The transdifferentiation of fibroblasts into myofibroblasts is modulated by cancer cell-derived cytokines, such as TGF- $\beta$  [76]. Myofibroblasts are present at the invasive front in cancer [77]. When isolated from colon cancer, they stimulate invasion of colon tumor cells. Tenascin C, secreted by myofibroblasts, is necessary for invasion driven by hepatocyte growth factor (HGF) [78]. Alternatively spliced tenascin C fibronectin domains have been reported in tumors and tumor-associated stromal cells [65, 79, 80]. It remains to be elucidated whether there is any functional difference among tenascin C variants, or between melanoma- and stromal cell-derived tenascin C.

#### Osteopontin in Melanoma

Osteopontin is a secreted, phosphorylated acidic glycoprotein that is involved in different physiological and patho-

logical events including regulation of inflammation, tissue remodeling, and cell survival [81]. Osteopontin interacts with receptors via arginine-glycine-aspartate (RGD)- and non-RGD containing adhesive domains, in addition to binding to components of the structural ECM [82]. Such receptors include integrins and variant forms of CD44. Osteopontin mediates cell-matrix interactions and cellular signaling by binding with these receptors.

Osteopontin is expressed in a variety of tissues, including vascular smooth muscle, activated macrophages, lymphocytes, breast and prostate cancer, osteosarcoma, glioblastoma, squamous cell carcinoma and melanoma [83]. Elevated osteopontin levels in serum can be a sensitive and specific marker in predicting disease progression in head and neck, renal, gastric, hepatocellular, lung, and pancreatic cancers, and melanoma [84, 85].

Human melanoma cells acquire osteopontin expression in the early steps of invasion [40]. Like tenascin C, osteopontin expression is robustly increased in response to TGF- $\beta$  [86]. Additionally, osteopontin was more abundant at both the mRNA and protein levels in tumor suppressor phosphatase and tensin homolog (PTEN) mutants which occur in some melanomas [87], indicating that osteopontin acts downstream of the phosphatidylinositol 3-kinase (PI3K) pathway.

The biological significance of osteopontin in melanoma progression has been studied using osteopontin deficient mice. In an experimental metastasis assay using B16 mouse melanoma cells, the number of tumors established in bone and lung was significantly reduced in osteopontin-deficient mice compared with wild-type mice [88]. Because B16 cells do not express osteopontin by themselves, the data suggests that host-derived osteopontin promotes metastasis formation. Osteopontin upregulates the migratory activity of B16 cells in a mitogen-activated protein kinase/extracellular signal-regulated kinase (MAPK/ERK) dependent manner [89]. Osteopontin stimulates invasion of murine melanoma cell lines and upregulates MMP-2 and MMP-9 via nuclear factor (NF)- $\kappa$ B activation [90, 91]. Increased expression and activity of the Src family of protein tyrosine kinases is observed in human melanoma cell lines and in melanoma tumors *in vivo* [27, 92]. Osteopontin activates c-Src in an integrin  $\alpha$ v-dependent manner [93]. Because Src kinase activity is required for integrin  $\alpha$ v $\beta$ 3-mediated activation of NF- $\kappa$ B in endothelial cells [94], this axis may also be responsible for MMPs upregulation in melanoma.

Like other matricellular proteins, there are multiple isoforms of osteopontin [95–98], suggesting it might have diverse physiological roles depending on the structural characteristics of each isoform. To date, it is not clear whether melanoma-derived osteopontin has a distinctive conformation and/or function in comparison to host-derived osteopontin.

## Conclusions and Future Directions

We are beginning to understand the functions of CCN3 production by melanocytes. CCN3 affects two fundamental features of melanocytic physiology—inhibiting melanocyte proliferation and stimulating adhesion to collagen type IV, the main component of the basement membrane. CCN3 is expressed at low levels in melanoma in contrast to other matricellular proteins, such as SPARC, osteopontin and tenascin C, which are upregulated and act as de-adhesive molecules which antagonize focal adhesion. CCN3 is the first member of the matricellular family found to be downregulated in advanced melanoma, and lack of CCN3 correlates with an invasive phenotype. The changes in matricellular protein expression in melanoma are reminiscent of the E-cadherin to N-cadherin class switch, allowing melanoma cells to escape keratinocyte control.

Recent findings describing novel roles for matricellular proteins in stem cell biology may provide clues as to their functions in tumor development. During the last decade, abundant evidence has been presented that stem cell-like populations exist in many types of cancer (cancer stem cells: CSCs) [99, 100]. Although CSCs are small subpopulations within a tumor, they are able to self-renew and re-initiate the tumor. Melanomas also contain CSCs that are highly tumorigenic and able to differentiate into multiple cell lineages [101]. The concept of CSCs could guide research in finding novel and effective therapeutic targets. In an organism, the specific microenvironment, or niche, plays a critical role in maintenance of stem cells [102]. It is not clear whether CSCs are dependent upon the same niche as normal stem cells [103]. One may hypothesize that the malignant tumor provides a specific niche for CSCs such that they no longer require the normal stem cell niche.

Several studies have determined that matricellular proteins are critical elements in the stem cell niche, not only to control the stem cell pool but to also regulate stem cell fate [104]. For example, osteopontin contributes to hematopoietic stem cell regulation by suppressing stem cell proliferation, thereby limiting the number of stem cells under homeostatic conditions. Tenascin C appears to regulate neuronal stem cell fate by modulating stem cell sensitivity to fibroblast growth factor 2 and bone morphogenetic protein 4 [105]. Considering that stem cell-like populations exist in many types of cancer including melanoma, the search is ongoing whether tumor-derived matricellular proteins provide a specific niche for stem cell-like populations in melanoma and other tumors.

In conclusion, further studies elucidating the mechanism underlying the matricellular protein switch are likely to reveal therapeutic targets for the prevention of melanoma progression.

**Open Access** This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

## References

1. Haass NK, Smalley KS, Li L et al (2005) Adhesion, migration and communication in melanocytes and melanoma. *Pigment Cell Res* 18:150–159
2. Bornstein P (1995) Diversity of function is inherent in matricellular proteins: an appraisal of thrombospondin 1. *J Cell Biol* 130:503–506
3. Bornstein P, Sage EH (2002) Matricellular proteins: extracellular modulators of cell function. *Curr Opin Cell Biol* 14:608–616
4. Brigstock DR (1999) The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. *Endocr Rev* 20:189–206
5. Perbal B (2004) CCN proteins: multifunctional signalling regulators. *Lancet* 363:62–64
6. Fukunaga-Kalabis M, Martinez G, Liu ZJ et al (2006) CCN3 controls 3D spatial localization of melanocytes in the human skin through DDR1. *J Cell Biol* 175:563–569
7. Valyi-Nagy IT, Hirka G, Jensen PJ et al (1993) Undifferentiated keratinocytes control growth, morphology, and antigen expression of normal melanocytes through cell-cell contact. *Lab Invest* 69:152–159
8. Shih IM, Elder DE, Hsu MY et al (1994) Regulation of Mel-CAM/MUC18 expression on melanocytes of different stages of tumor progression by normal keratinocytes. *Am J Pathol* 145:837–845
9. Coffey RJ Jr, Derynck R, Wilcox JN et al (1987) Production and auto-induction of transforming growth factor- $\alpha$  in human keratinocytes. *Nature* 328:817–820
10. Luger TA, Schwarz T (1990) Evidence for an epidermal cytokine network. *J Invest Dermatol* 95:100S–104S
11. Imokawa G (2004) Autocrine and paracrine regulation of melanocytes in human skin and in pigmentary disorders. *Pigment Cell Res* 17:96–110
12. Perbal B (2001) NOV (nephroblastoma overexpressed) and the CCN family of genes: structural and functional issues. *Mol Pathol* 54:57–79
13. Perbal B (1999) Nuclear localisation of NOVH protein: a potential role for NOV in the regulation of gene expression. *Mol Pathol* 52:84–91
14. Bleau AM, Planque N, Lazar N et al (2007) Antiproliferative activity of CCN3: involvement of the C-terminal module and post-translational regulation. *J Cell Biochem* 101:1475–1491
15. Joliot V, Martinerie C, Dambrine G et al (1992) Proviral rearrangements and overexpression of a new cellular gene (nov) in myeloblastosis-associated virus type 1-induced nephroblastomas. *Mol Cell Biol* 12:10–21
16. Fu CT, Bechberger JF, Ozog MA et al (2004) CCN3 (NOV) interacts with connexin43 in C6 glioma cells: possible mechanism of connexin-mediated growth suppression. *J Biol Chem* 279:36943–36950
17. Benini S, Perbal B, Zambelli D et al (2005) In Ewing's sarcoma CCN3(NOV) inhibits proliferation while promoting migration and invasion of the same cell type. *Oncogene* 24:4349–4361
18. Gellhaus A, Dong X, Propson S et al (2004) Connexin43 interacts with NOV: a possible mechanism for negative regulation of cell growth in choriocarcinoma cells. *J Biol Chem* 279:36931–36942

19. Matic M, Evans WH, Brink PR et al (2002) Epidermal stem cells do not communicate through gap junctions. *J Invest Dermatol* 118:110–116
20. Sonnenberg A, Gehlsen KR, Aumailley M et al (1991) Isolation of alpha 6 beta 1 integrins from platelets and adherent cells by affinity chromatography on mouse laminin fragment E8 and human laminin pepsin fragment. *Exp Cell Res* 197:234–244
21. Albelda SM, Mette SA, Elder DE et al (1990) Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. *Cancer Res* 50:6757–6764
22. Hara M, Yaar M, Tang A et al (1994) Role of integrins in melanocyte attachment and dendricity. *J Cell Sci* 107(Pt 10):2739–2748
23. Krengel S, Stark I, Geuchen C et al (2005) Selective down-regulation of the alpha6-integrin subunit in melanocytes by UVB light. *Exp Dermatol* 14:411–419
24. Vogel W (1999) Discoidin domain receptors: structural relations and functional implications. *FASEB J* 13(Suppl):S77–82
25. Ongusaha PP, Kim JI, Fang L et al (2003) p53 induction and activation of DDR1 kinase counteract p53-mediated apoptosis and influence p53 regulation through a positive feedback loop. *EMBO J* 22:1289–1301
26. Tong X, O’Kelly J, Xie D et al (2004) Cyr61 suppresses the growth of non-small-cell lung cancer cells via the beta-catenin-c-myc-p53 pathway. *Oncogene* 23:4847–4855
27. Lin CG, Leu SJ, Chen N et al (2003) CCN3 (NOV) is a novel angiogenic regulator of the CCN protein family. *J Biol Chem* 278:24200–24208
28. Hsu MY, Meier FE, Nesbit M et al (2000) E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. *Am J Pathol* 156:1515–1525
29. Sakamoto K, Yamaguchi S, Ando R et al (2002) The nephroblastoma overexpressed gene (NOV/ccn3) protein associates with Notch1 extracellular domain and inhibits myoblast differentiation via notch signaling pathway. *J Biol Chem* 277:29399–29405
30. Maillard M, Cadot B, Ball RY et al (2001) Differential expression of the ccn3 (nov) proto-oncogene in human prostate cell lines and tissues. *Mol Pathol* 54:275–280
31. Glukhova L, Angevin E, Lavielle C et al (2001) Patterns of specific genomic alterations associated with poor prognosis in high-grade renal cell carcinomas. *Cancer Genet Cytogenet* 130:105–110
32. Manara MC, Perbal B, Benini S et al (2002) The expression of ccn3(nov) gene in musculoskeletal tumors. *Am J Pathol* 160:849–859
33. Perbal B, Brigstock DR, Lau LF (2003) Report on the second international workshop on the CCN family of genes. *Mol Pathol* 56:80–85
34. Gupta N, Wang H, McLeod TL et al (2001) Inhibition of glioma cell growth and tumorigenic potential by CCN3 (NOV). *Mol Pathol* 54:293–299
35. McCallum L, Price S, Planque N et al (2006) A novel mechanism for BCR-ABL action: stimulated secretion of CCN3 is involved in growth and differentiation regulation. *Blood* 108:1716–1723
36. Laurent M, Martinerie C, Thibout H et al (2003) NOVH increases MMP3 expression and cell migration in glioblastoma cells via a PDGFR-alpha-dependent mechanism. *FASEB J* 17:1919–1921
37. Fukunaga-Kalabis M, Martinez G, Telson SM, et al (2008) Downregulation of CCN3 expression as a potential mechanism for melanoma progression. *Oncogene* DOI [10.1038/sj.onc.1210896](https://doi.org/10.1038/sj.onc.1210896)
38. Clark WH (1991) Tumour progression and the nature of cancer. *Br J Cancer* 64:631–644
39. Haass NK, Smalley KS, Herlyn M (2004) The role of altered cell–cell communication in melanoma progression. *J Mol Histol* 35:309–318
40. Zhou Y, Dai DL, Martinka M et al (2005) Osteopontin expression correlates with melanoma invasion. *J Invest Dermatol* 124:1044–1052
41. Herlyn M, Graeven U, Speicher D et al (1991) Characterization of tenascin secreted by human melanoma cells. *Cancer Res* 51:4853–4858
42. Massi D, Franchi A, Borgognoni L et al (1999) Osteonectin expression correlates with clinical outcome in thin cutaneous malignant melanomas. *Hum Pathol* 30:339–344
43. Hsu MY, Wheelock MJ, Johnson KR et al (1996) Shifts in cadherin profiles between human normal melanocytes and melanomas. *J Invest Dermatol Symp Proc* 1:188–194
44. Welch DR, Sakamaki T, Pioquinto R et al (2000) Transfection of constitutively active mitogen-activated protein/extracellular signal-regulated kinase confers tumorigenic and metastatic potentials to NIH3T3 cells. *Cancer Res* 60:1552–1556
45. Denkert C, Siegert A, Leclere A et al (2002) An inhibitor of stress-activated MAP-kinases reduces invasion and MMP-2 expression of malignant melanoma cells. *Clin Exp Metastasis* 19:79–85
46. Tanimura S, Asato K, Fujishiro SH et al (2003) Specific blockade of the ERK pathway inhibits the invasiveness of tumor cells: down-regulation of matrix metalloproteinase-3/-9/-14 and CD44. *Biochem Biophys Res Commun* 304:801–806
47. Planque N, Long Li C, Saule S et al (2006) Nuclear addressing provides a clue for the transforming activity of amino-truncated CCN3 proteins. *J Cell Biochem* 99:105–116
48. Vallacchi V, Daniotti M, Ratti F et al (2008) CCN3/nephroblastoma overexpressed matricellular protein regulates integrin expression, adhesion, and dissemination in melanoma. *Cancer Res* 68:715–723
49. Dvorak HF (1986) Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med* 315:1650–1659
50. Kalluri R, Zeisberg M (2006) Fibroblasts in cancer. *Nat Rev Cancer* 6:392–401
51. Bradshaw AD, Sage EH (2001) SPARC, a matricellular protein that functions in cellular differentiation and tissue response to injury. *J Clin Invest* 107:1049–1054
52. Bornstein P (2001) Thrombospondins as matricellular modulators of cell function. *J Clin Invest* 107:929–934
53. Sturm RA, Satyamoorthy K, Meier F et al (2002) Osteonectin/SPARC induction by ectopic beta(3) integrin in human radial growth phase primary melanoma cells. *Cancer Res* 62:226–232
54. Framson PE, Sage EH (2004) SPARC and tumor growth: where the seed meets the soil? *J Cell Biochem* 92:679–690
55. Robert G, Gaggioli C, Bailet O et al (2006) SPARC represses E-cadherin and induces mesenchymal transition during melanoma development. *Cancer Res* 66:7516–7523
56. Smit DJ, Gardiner BB, Sturm RA (2007) Osteonectin down-regulates E-cadherin, induces osteopontin and focal adhesion kinase activity stimulating an invasive melanoma phenotype. *Int J Cancer* 121:2653–2660
57. Alonso SR, Tracey L, Ortiz P et al (2007) A high-throughput study in melanoma identifies epithelial-mesenchymal transition as a major determinant of metastasis. *Cancer Res* 67:3450–3460
58. Alvarez MJ, Prada F, Salvatierra E et al (2005) Secreted protein acidic and rich in cysteine produced by human melanoma cells modulates polymorphonuclear leukocyte recruitment and anti-tumor cytotoxic capacity. *Cancer Res* 65:5123–5132
59. Prada F, Benedetti LG, Bravo AI et al (2007) SPARC endogenous level, rather than fibroblast-produced SPARC or



- stroma reorganization induced by SPARC, is responsible for melanoma cell growth. *J Invest Dermatol* 127:2618–2628
60. Sage EH, Reed M, Funk SE et al (2003) Cleavage of the matricellular protein SPARC by matrix metalloproteinase 3 produces polypeptides that influence angiogenesis. *J Biol Chem* 278:37849–37857
  61. Iruela-Arispe ML, Lane TF, Redmond D et al (1995) Expression of SPARC during development of the chicken chorioallantoic membrane: evidence for regulated proteolysis in vivo. *Mol Biol Cell* 6:327–343
  62. Yan Q, Weaver M, Perdue N et al (2005) Matricellular protein SPARC is translocated to the nuclei of immortalized murine lens epithelial cells. *J Cell Physiol* 203:286–294
  63. Schultz C, Lemke N, Ge S et al (2002) Secreted protein acidic and rich in cysteine promotes glioma invasion and delays tumor growth in vivo. *Cancer Res* 62:6270–6277
  64. Koblinski JE, Kaplan-Singer BR, VanOsdol SJ et al (2005) Endogenous osteonectin/SPARC/BM-40 expression inhibits MDA-MB-231 breast cancer cell metastasis. *Cancer Res* 65:7370–7377
  65. Orend G, Chiquet-Ehrismann R (2006) Tenascin-C induced signaling in cancer. *Cancer Lett* 244:143–163
  66. Murphy-Ullrich JE (2001) The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *J Clin Invest* 107:785–790
  67. Chiquet-Ehrismann R, Kalla P, Pearson CA et al (1988) Tenascin interferes with fibronectin action. *Cell* 53:383–390
  68. Tuominen H, Kallioinen M (1994) Increased tenascin expression in melanocytic tumors. *J Cutan Pathol* 21:424–429
  69. Breslow A (1970) Thickness, cross-sectional areas and depth of invasion in the prognosis of cutaneous melanoma. *Ann Surg* 172:902–908
  70. Kaariainen E, Nummela P, Soikkeli J et al (2006) Switch to an invasive growth phase in melanoma is associated with tenascin-C, fibronectin, and procollagen-I forming specific channel structures for invasion. *J Pathol* 210:181–191
  71. Bidard FC, Pierga JY, Vincent-Salomon A et al (2008) A “class action” against the microenvironment: do cancer cells cooperate in metastasis? *Cancer Metastasis Rev* 27:5–10
  72. Sarkar S, Nuttall RK, Liu S et al (2006) Tenascin-C stimulates glioma cell invasion through matrix metalloproteinase-12. *Cancer Res* 66:11771–11780
  73. Ilunga K, Nishiura R, Inada H et al (2004) Co-stimulation of human breast cancer cells with transforming growth factor-beta and tenascin-C enhances matrix metalloproteinase-9 expression and cancer cell invasion. *Int J Exp Pathol* 85:373–379
  74. Galoian KA, Garamszegi N, Garamszegi SP et al (2007) Molecular mechanism of tenascin-C action on matrix metalloproteinase-1 invasive potential. *Exp Biol Med* (Maywood) 232:515–522
  75. Desmouliere A, Guyot C, Gabbiani G (2004) The stroma reaction myofibroblast: a key player in the control of tumor cell behavior. *Int J Dev Biol* 48:509–517
  76. De Wever O, Mareel M (2003) Role of tissue stroma in cancer cell invasion. *J Pathol* 200:429–447
  77. De Wever O, Mareel M (2002) Role of myofibroblasts at the invasion front. *Biol Chem* 383:55–67
  78. De Wever O, Nguyen QD, Van Hoorde L et al (2004) Tenascin-C and SF/HGF produced by myofibroblasts in vitro provide convergent pro-invasive signals to human colon cancer cells through RhoA and Rac. *FASEB J* 18:1016–1018
  79. Derr LB, Chiquet-Ehrismann R, Gandour-Edwards R et al (1997) The expression of tenascin-C with the AD1 variable repeat in embryonic tissues, cell lines and tumors in various vertebrate species. *Differentiation* 62:71–82
  80. Hindermann W, Berndt A, Borsi L et al (1999) Synthesis and protein distribution of the unspliced large tenascin-C isoform in oral squamous cell carcinoma. *J Pathol* 189:475–480
  81. Denhardt DT, Noda M, O’Regan AW et al (2001) Osteopontin as a means to cope with environmental insults: regulation of inflammation, tissue remodeling, and cell survival. *J Clin Invest* 107:1055–1061
  82. Giachelli CM, Steitz S (2000) Osteopontin: a versatile regulator of inflammation and biomineralization. *Matrix Biol* 19:615–622
  83. Rangaswami H, Bulbule A, Kundu GC (2006) Osteopontin: role in cell signaling and cancer progression. *Trends Cell Biol* 16:79–87
  84. Wai PY, Kuo PC (2008) Osteopontin: regulation in tumor metastasis. *Cancer Metastasis Rev* 27:103–118
  85. Rangel J, Nosrati M, Torabian S et al (2008) Osteopontin as a molecular prognostic marker for melanoma. *Cancer* 112:144–150
  86. Javelaud D, Mohammad KS, McKenna CR et al (2007) Stable overexpression of Smad7 in human melanoma cells impairs bone metastasis. *Cancer Res* 67:2317–2324
  87. Packer L, Pavey S, Parker A et al (2006) Osteopontin is a downstream effector of the PI3-kinase pathway in melanomas that is inversely correlated with functional PTEN. *Carcinogenesis* 27:1778–1786
  88. Nemoto H, Rittling SR, Yoshitake H et al (2001) Osteopontin deficiency reduces experimental tumor cell metastasis to bone and soft tissues. *J Bone Miner Res* 16:652–659
  89. Hayashi C, Rittling S, Hayata T et al (2007) Serum osteopontin, an enhancer of tumor metastasis to bone, promotes B16 melanoma cell migration. *J Cell Biochem* 101:979–986
  90. Philip S, Bulbule A, Kundu GC (2001) Osteopontin stimulates tumor growth and activation of promatrix metalloproteinase-2 through nuclear factor-kappa B-mediated induction of membrane type 1 matrix metalloproteinase in murine melanoma cells. *J Biol Chem* 276:44926–44935
  91. Rangaswami H, Bulbule A, Kundu GC (2004) Nuclear factor-inducing kinase plays a crucial role in osteopontin-induced MAPK/IkappaBalpha kinase-dependent nuclear factor kappaB-mediated promatrix metalloproteinase-9 activation. *J Biol Chem* 279:38921–38935
  92. Homsy J, Cubitt C, Daud A (2007) The Src signaling pathway: a potential target in melanoma and other malignancies. *Expert Opin Ther Targets* 11:91–100
  93. Chellaiah M, Fitzgerald C, Filardo EJ et al (1996) Osteopontin activation of c-src in human melanoma cells requires the cytoplasmic domain of the integrin alpha v-subunit. *Endocrinology* 137:2432–2440
  94. Courter DL, Lomas L, Scatena M et al (2005) Src kinase activity is required for integrin alphaVbeta3-mediated activation of nuclear factor-kappaB. *J Biol Chem* 280:12145–12151
  95. Kiefer MC, Bauer DM, Barr PJ (1989) The cDNA and derived amino acid sequence for human osteopontin. *Nucleic Acids Res* 17:3306
  96. Young MF, Kerr JM, Termine JD et al (1990) cDNA cloning, mRNA distribution and heterogeneity, chromosomal location, and RFLP analysis of human osteopontin (OPN). *Genomics* 7:491–502
  97. Saitoh Y, Kuratsu J, Takeshima H et al (1995) Expression of osteopontin in human glioma. Its correlation with the malignancy. *Lab Invest* 72:55–63
  98. Kon S, Maeda M, Segawa T et al (2000) Antibodies to different peptides in osteopontin reveal complexities in the various secreted forms. *J Cell Biochem* 77:487–498

99. Reya T, Morrison SJ, Clarke MF et al (2001) Stem cells, cancer, and cancer stem cells. *Nature* 414:105–111
100. Wicha MS, Liu S, Dontu G (2006) Cancer stem cells: an old idea—a paradigm shift. *Cancer Res* 66:1883–1890 (discussion 1895–1886)
101. Fang D, Nguyen TK, Leishear K et al (2005) A tumorigenic subpopulation with stem cell properties in melanomas. *Cancer Res* 65:9328–9337
102. Moore KA, Lemischka IR (2006) Stem cells and their niches. *Science* 311:1880–1885
103. Li L, Neaves WB (2006) Normal stem cells and cancer stem cells: the niche matters. *Cancer Res* 66:4553–4557
104. Nilsson SK, Johnston HM, Whitty GA et al (2005) Osteopontin, a key component of the hematopoietic stem cell niche and regulator of primitive hematopoietic progenitor cells. *Blood* 106:1232–1239
105. Garcion E, Halilagic A, Faissner A et al (2004) Generation of an environmental niche for neural stem cell development by the extracellular matrix molecule tenascin C. *Development* 131:3423–3432