Chapter 17
The Surface Transplantation Model to Study the Tumor–Host Interface

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Abstract The tumor is a complex system comprising neoplastic genetically altered cells and a tumor stroma composed of remodeled extracellular matrix, newly formed vessels, and infiltrating host cells. The development of a cancer is a progressive multistep process in which neoplastic cells progress to malignancy by activating their microenvironment and by responding to the tumor-supporting cues of the surrounding tissue. Because of the recently recognized importance of a permissive stroma for tumor development and invasion, the host compartment is now viewed as an interesting new target for tumor therapy. Among positive regulators contributing to the elaboration of this permissive stroma are growth factors, cytokines/chemokines, proteases, and their inhibitors. The present review summarizes what we learned during the last decade on the contribution of these factors at the tumor–host interface by exploiting a useful in vivo surface transplantation model of skin carcinomas.

Introduction

Tumors are not only composed of neoplastic cells, but they are also heterogeneous, structurally complex, and result from an evolving crosstalk between tumor cells and different host cell types. Genetic alterations in tumor cells are essential for tumor progression but not sufficient to generate malignant tumors. Indeed, the stromal

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environment is required to create a permissive soil for the invasion of the seed, the genetically altered tumor cells. Since the "seed and soil" hypothesis proposed by Paget in 1889 (Paget 1889), the importance of tumor–stroma interactions have been documented (Hanahan and Weinberg 2000, Stetler-Stevenson and Braylan 2001, Fidler 2003, Carmeliet 2005, Noel et al. 2008). Indeed, the stroma of malignant tumors resembles the granulation tissue of a healing wound (Dvorak 1986, Mueller and Fusenig 2002) and alterations of the stromal environment (Zigrino et al. 2005) include enhanced vascularization through angiogenesis and vasculogenesis (Carmeliet 2005, Li et al. 2006), modified extracellular matrix (ECM) composition, recruitment of fibroblastic cells (Kalluri and Zeisberg 2006) and inflammatory cells, and unbalanced protease activities (Folgueras et al. 2004, Zigrino et al. 2005). Consequently, the evolution of tumor xenografts in mice is known to depend on the presence of several host cells including fibroblasts (Noel et al. 1994, Noel et al. 1998, Zhang et al. 2006), adipocytes (Kuperwasser et al. 2004, Andarawewa et al. 2005) immune as well as inflammatory cells (Coussens and Werb 2001), and endothelial cells (Skobe et al. 1997, Vosseler et al. 2005).

In addition to inflammation, the acquisition of an angiogenic phenotype is viewed as a prerequisite for tumor progression. Neovascularization is crucial for sustained tumor growth since it allows oxygenation and nutrient perfusion of the tumor as well as removal of waste products (Carmeliet and Jain 2000). Additionally, vascular endothelial cells can stimulate tumor growth in a paracrine manner by inducing tumor proliferation and invasion. Finally, increased angiogenesis coincides with increased tumor cell entry into the blood circulation and thus facilitates metastasis. Several lines of evidence indicate that induction of angiogenesis precedes the formation of malignant tumors, suggesting that angiogenesis may be rate limiting not only for tumor expansion but also for the onset of malignancy (Skobe and Fusenig 1998, Mueller and Fusenig 2004). As a consequence of the recognition of the essential role of the tumor–stroma interface for tumor progression, the tumor microenvironment has emerged as a new putative target for tumor therapy. Extensive research during more than 30 years led to the entry of anti-VEGF (vascular endothelial growth factor) therapeutics in clinical practice for the therapy of cancers (Duda et al. 2007). Bevacizumab (Avastin®, Genentech Inc.), an anti-VEGF antibody, was the first anti-angiogenic compound approved in 2004 by the US Food and Drug Administration (Carmeliet 2005, Ferrara and Kerbel 2005). However, despite the remarkable rapid clinical development of anti-VEGF agents, a growing body of preclinical evidence suggests that other angiogenic pathways are as important in disease progression and might explain the resistance appearing after anti-VEGF therapy (Duda et al. 2007).

In this context, it is essential to keep in mind that the modifications of stromal features are controlled by tumor cells themselves, depending on their degree of aggressiveness and invasiveness. Tumor cells can regulate the elaboration of a permissive stromal environment via the aberrant expression of angiogenic factors (VEGF, placental-like growth factor or PlGF, platelet-derived growth factor or PDGF), proteases (matrix metalloproteinases or MMPs, serine proteases, cathepsins), and chemotactic proteins (stromal cell-derived factor 1 or SDF1α, macrophage chemotactic protein-1 or MCP1) (Bergers and Benjamin 2003).

These upregulated factors disrupt normal tissue homeostasis and act in a paracrine manner to induce stromal reactions such as angiogenic and inflammatory responses (Coussens and Werb 2001, Balkwill and Coussens 2004, Mueller and Fusenig 2004). The recruited host cells are important producers of growth factors, cytokines, chemokines, and proteases, all essential for ECM remodeling, cell migration, and angiogenesis (Benelli et al. 2001, Balkwill and Coussens 2004). Proteases contribute to the remodeling of ECM, promoting host cell migration (inflammation and angiogenesis) and tumor cell invasion. Proteases act not only by disrupting physiological barriers such as basement membranes but importantly also by releasing growth and chemotactic factors from the ECM and demasking cryptic domains of matrix components (Kalluri 2003). In addition, they are key regulators of the shedding, activation, and/or degradation of cell surface molecules including adhesion molecules, mediators of apoptosis, receptors of chemokines/cytokines, and intercellular junction proteins (Cauwe et al. 2007).

The role of proteases in the regulation of angiogenesis and tumor progression made them initially very desirable as therapeutic targets. However, the failure of clinical trials with broad-spectrum MMP inhibitors in cancer (Coussens et al. 2002) (*see* also Chap. 36) made very clear that the role of proteases during tumor growth and progression as well as stromal activation and angiogenesis is much more complex than initially expected (Matrisian and Lopez-Otin 2007). Therefore, the development of new therapies requires an in-depth understanding of the complex interactions established between host and tumor. The functional role of the stroma is difficult to delineate in classical in vivo models of spontaneous or transplanted tumors, due to the intermingled close association of tumor and stroma elements. Therefore, the analysis of tumor–stroma interactions and their role in tumor development requires experimental in vivo systems reflecting different tumor stages. To fulfill those requirements, an in vivo model has been set up to study tumor–stroma interactions: the surface transplantation model (Fusenig et al. 1983).

Surface Tranplants of Squamous Cell Carcinoma of the Skin

The surface transplantation model, which was initially developed to study the interactions of normal epithelial and stromal cells and their impact on growth and differentiation (Fusenig 1992), allows the complete reconstitution of a skin epithelium under the influence of the connective tissue environment, without direct contact between epithelial and stromal cells (schematically shown in Fig. 17.1a). In this model, keratinocytes (of mouse or human origin) are precultured on a 2–3 mm thick type-I collagen gel mounted between two concentric Teflon rings. When a confluent monolayer has formed, the culture is covered by a silicone transplantation chamber and transplanted in toto onto the back muscle fascia of mice where it is held in place by fixing it by wound clips with the surrounding mouse skin. Although separated from the host stroma by the collagen matrix, the grafted cells rapidly develop into highly proliferative stratified epithelia (Fig. 17.1b, e)

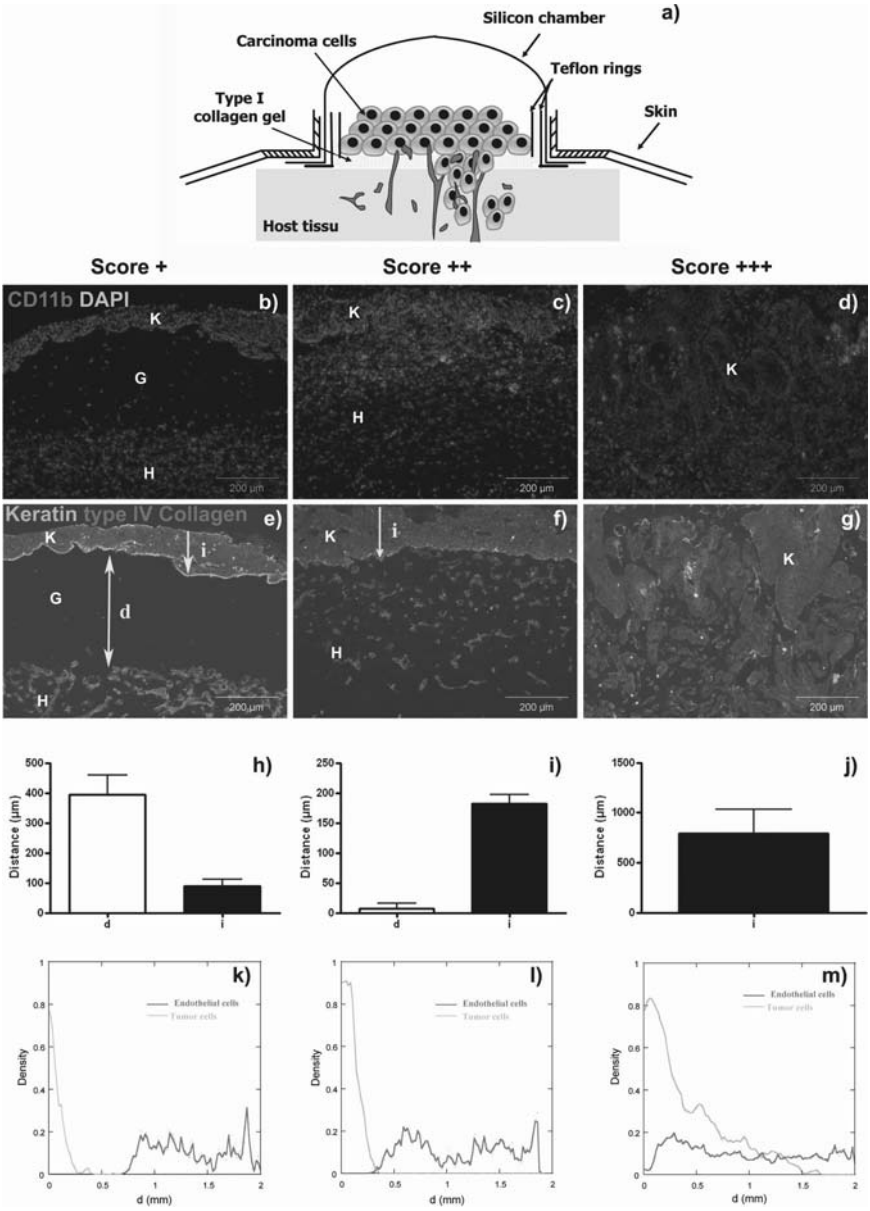

Fig. 17.1 Representation of skin carcinoma cell invasion in the surface transplantation model. **a** Schematic representation of the model: keratinocytes are cultured on the type-I collagen gel, mounted in concentric teflon rings and covered with a hat-shaped silicone chamber. **b–g** Different stages of tumor invasion (score +, ++, +++) are observed after 1 (**b, e**), 2 (**c, f**), or 3 (**d, g**) weeks of transplantation. After 1 week of transplantation, tumor cells proliferate to form a multilayered epithelium, and few inflammatory cells infiltrate the collagen gel (**b**). The collagen gel is

within 1–3 weeks. The collagen gel is gradually replaced by a highly vascularized granulation tissue, which eventually gets in close contact to the epithelium (Fig. 17.1c, d, f, g). Therefore, this model recapitulates different steps of skin carcinoma progression, mimics the microenvironment of a developing skin carcinoma, and allows a kinetic analysis of tumor–stroma interactions during tumor development and angiogenic switch (Mueller and Fusenig 2004).

The stromal compartment of malignant tumors is important and continuous interaction between tumor and stromal cells is prerequisite for carcinoma development and progression. One important finding of studies using the transplantation chamber assay is that although tumor cells rapidly proliferate forming multilayered epithelia on top of the gel, invasive growth, the hallmark of malignancy, does not manifest until the vascularized granulation tissue has replaced the gel and approached the tumor cells. Thus, a close association and interaction between tumor and stromal tissue is obviously needed for tumor invasion (Skobe et al. 1997; Mueller and Fusenig 2002). This sequential course of stromal activation and tumor invasion indicates that rapid interactions between tumor and host cells occur on transplantation, resulting first in activation of stromal tissue. However, such an early sequence of events became apparent only by using the matrix-inserted transplantation assay, in which a collagen gel is interposed between tumor cells and stromal compartments. This particular transplantation model displays several crucial advantages. The collagen gel provides an appropriate substratum for tumor cell attachment and serves as a temporal "barrier" preventing immediate contact between grafted tumor cells and host cells. However, it allows a dialogue between these cells via diffusible factors (growth factors, angiogenic factors, cytokines/chemokines) allowing to characterize the kinetics of the different stromal responses in depth (infiltration of inflammatory cells, fibroblastic cells, and angiogenesis). Indeed, the differential tumorigenic potential of cells is even more evident in this surface transplant system in which benign clones form slightly dysplastic keratinizing epithelia, while malignant cells develop into invasive carcinoma (Boukamp et al. 1990, Breitkreutz et al. 1991). Interestingly, the onset strength of the stromal reaction clearly correlates with the stage of malignancy, being later and weakest in transplants of benign and earliest and strongest in those of metastatic cells.

Fig. 17.1 (Continued) progressively replaced by a granulation tissue at 2 weeks (**c**), leading to a vascularized tumor at 3 weeks (**d**). **b–d**: Immunostaining of CD11b, a marker of granulocytes and monocytes/macrophages (K: tumor cells; G: collagen gel; H: host tissue). **e–g** Immunostaining of keratinocytes (green) and vessels (red). **e** A score of + is attributed when blood vessels remain below the collagen gel or particularly the matrix. **f** When blood vessels get into close contact with malignant epithelial layer, a score of ++ is assigned. **g** The score is +++ when tumor and blood vessels are intermingled. **h–j** Tumor invasion can be quantified by manual measurements of the distance "*i*," between the top of tumor cell layer and the deepest front of tumor spread (yellow arrows) (**e, f**). In this system, vascularization is estimated by measuring the distance "*d*" separating tumor cells from the front blood vessel migration (**e**). Quantification by computerized image analysis consists in determining the distribution of tumor/endothelial cell density as a function of the distance to the upper boundary of tumor (**k–m**). (*See also* Color Insert I)

Methods to Quantify Tumor Cell Invasion

Different methods are used to quantify tumor cell invasion and the extent of the angiogenic response (Fig. 17.1). The more simple appreciation of tumor development and progression is a semiquantitative scoring of cell invasion (Fig. 17.1). Score + to +++ is assigned according to the infiltration of blood vessels into the collagen gels toward tumor cells (Bajou et al. 1998, 2001, Jost et al. 2006) (Fig. 17.1b–g). A more objective method of quantification relies on the manual measurements of (1) tumor invasion by determining the distance "*i*" between the top of the tumor cell layer and the deepest front of tumor spread (Fig. 17.1d, e) and (2) angiogenesis by estimating the distance "*d*" separating the tumor cells from the front of blood vessel migration (Fig. 17.1d) (Bajou et al. 2004). More recently, an original image analysis algorithm for computerized processing has been set up (Jost et al. 2006, Blacher et al. 2008). This method determines the tumor/endothelial cell density as a function of the distance to the upper boundary of the tumor layer (Fig. 17.1). It provides more information relating to the morphology of the studied structures, and can precisely estimate the intermingling between tumor cells and blood vessels (Jost et al. 2006). Another method to quantify tumor cell invasion and angiogenesis is the morphometric analysis using analySIS software (Olympus). Using this tool, tumor transplants immunostained for endothelial cell and tumor cell markers were photographed and divided into two major compartments, 300 μm below and 500 μm within the tumor, respectively. The CD31-stained areas were calculated by analySIS software for these two compartments, leading to a quantitative estimation for tumor cell invasion (within tumor) and for mean vessel density (below tumor) (Vosseler et al. 2005, Obermueller et al. 2004). Interestingly, in addition to estimating the malignant features of different tumor cells, the surface model offers the possibility to determine the key molecular determinants of both tumor and host compartments.

Proteases and Inhibitors as Key Molecular Determinants of the Host Compartment

Different classes of proteases have been implicated during different stages of cancer progression. The principal classes of proteases involved are the MMPs, serine proteases, and cathepsins (van Hinsbergh et al. 2006). The functional association between MMPs and the plasminogen activator (PA)/plasmin systems, in particular the role of plasmin as a pro-MMP activator, has generated substantial attention in the context of both physiological and pathological tissue remodeling (Folgueras et al. 2004, Lee and Huang 2005, van Hinsbergh et al. 2006). Members of the MMP family and components of the PA system are coexpressed during development, tissue remodeling, tissue repair, but also in multiple diseases such as tumor invasion and metastasis (Lijnen 2001). These proteases control cell

proliferation, migration, and invasion by remodeling the ECM and releasing growth factors sequestered in the matrix. Furthermore, by cleaving extracellular components and shedding cell surface molecules, the proteases have been implicated in the activation and bioavailability of cytokines/chemokines, growth factor receptors, and integrins (Rakic et al. 2003, Egeblad and Werb 2002, Noel et al. 2004, Overall and Kleifeld 2006). Although it was initially believed that high production of proteases (MMPs and PA/plasmin system) came from neoplastic cells themselves, host stromal cells are now recognized as essential producers of proteases (Noel et al. 2008, Egeblad and Werb 2002).

The PA–plasmin system is a pericellular proteolytic system with pleiotropic functions in physiological and pathological tissue remodeling (Rakic et al. 2003, Durand et al. 2004, Noel et al. 2004, Binder et al. 2007) (*see* also Chaps. 10 and 11). It is a complex system of serine proteases, protease inhibitors, and protease receptors that governs the conversion of the abundant protease zymogen, plasminogen (Plg), into active plasmin. Activation of Plg appears to be strictly associated with the cell surface via the binding to specific receptors, as well as with other surfaces that present kinetically favorable circumstances for Plg activation, such as the fibrin thrombus (Myohanen and Vaheri 2004). Surface-generated plasmin is relatively protected from its primary physiological inhibitor α2-antiplasmin. Cell surface Plg activation by the two PAs, urokinase-type PA (uPA) and tissue-type PA (tPA), is regulated by two physiological inhibitors, Plg activator inhibitor-1 and -2 (PAI-1 and PAI-2), each forming a 1:1 complex with uPA and tPA. As an inhibitor of proteases, PAI-1 was initially viewed as an antiangiogenic and antitumoral factor. However, unexpected and novel results were obtained when the surface transplantation model using mouse malignant keratinocytes was applied to PAI-1-deficient mice. Indeed, the grafted mouse skin carcinoma cells failed to invade the stroma of PAI-1-deficient mice (Table 17.1) (Bajou et al. 1998, Bajou et al. 2001). These results were opposite to the initial hypothesis that a deficiency of protease inhibitor would enhance tumor growth and invasion. In these deficient mice, tumor cells can induce granulation tissue formation beneath the collagen gel and angiogenesis, but new blood vessels cannot reach the tumor layer. These results have been confirmed in other experimental models (Gutierrez et al. 2000, Devy et al. 2002, McMahon et al. 2001). The fact that the PAI-1 production by tumor cells cannot circumvent host cell deficiency, even at high concentration by transfecting malignant keratinocytes with PAI-1 cDNA (Bajou et al. 2004), clearly emphasizes the stroma tissue as the most important source of PAI-1 (Bajou et al. 1998, Maillard et al. 2005). Additionally, the local variation of PAI-1 concentration is very important for tumor development. Indeed, a dose-dependent proangiogenic effect of this inhibitor has been demonstrated in the SCC model (Bajou et al. 2004) and in another model of pathological angiogenesis, the choroidal neovascularization assay (Lambert et al. 2003a). The proangiogenic effect of PAI-1 in the surface transplantation model relies on its capacity to interact with uPA (Bajou et al. 2001). However, lack of uPA, tPA, or uPA receptor, as well as combined deficiencies of uPA and tPA, did not affect tumor angiogenesis, whereas lack of Plg reduced it (Table 17.1). Overall, these data indicate that plasmin proteolysis, even though

Table 17.1 Effects of serine protease gene deletion on tumor angiogenesis and invasion

KO mice	MMP cellular sources		In vivo transplantations scoring		MMP effect on tumor	References
	Host cells	Epithelial tumor cells	Tumor invasion	Angiogenesis		
Plg	−	−	+	+	Positive regulator	Bajou et al. 2001
uPA	+	+	+++	+++	/	Bajou et al. 2001
tPA	+	+	+++	+++	/	Bajou et al. 2001
uPA/tPA	+	+	+++	+++	/	Bajou et al. 2001
uPAR	+	+	+++	+++	/	Bajou et al. 2001
PAI-1 (C57Bl/6)	+	+	0	0	Positive regulator	Bajou et al. 1998; Bajou et al. 2001
PAI-1 (Rag-1 KO) (nu/nu)	+	+	0	0	Positive regulator	Maillard et al. 2005

Components of PA/plasmin system can be expressed by epithelial tumor cells (keratinocytes) or healthy stromal cells. Three weeks after tumor transplantation, angiogenesis and tumor invasion are determined by scoring + to +++ as described in Fig. 17.1. All corresponding wild-type (WT) mice presented an invasive and angiogenic score (+++).

essential, must be tightly controlled during tumor angiogenesis and other enzymes may, at least in part, contribute to the angiogenic phenotype (Bajou et al. 2001).

The MMPs constitute an additional large family of structurally related matrix-degrading proteases that have pivotal roles in development, tissue remodeling, and cancer. MMPs share a number of common structural and functional features (Lopez-Otin and Overall, 2002, Folgueras et al. 2004; Greenlee et al. 2007). All MMPs have essential zinc and calcium ions, are synthesized as zymogens, and are inhibited by endogenous inhibitors, such as α2-macroglobuline and specific MMP inhibitors or TIMPs (tissue inhibitors of metalloproteinases) that reversibly inhibit proteases in a 1:1 enzyme–inhibitor complex (Sternlicht and Werb 2001). MMPs have multiple domains that control their secretion, specificity, and substrate binding. Their function is tightly regulated at the level of gene expression, zymogen activation, enzyme activity, and cell-surface localization (Greenlee et al. 2007). The MMP protease family includes soluble enzymes secreted into the extracellular milieu and others associated with the cell surface (MT-MMPs, membrane-type metalloproteinases) (for review: Egeblad and Werb 2002, Folgueras et al. 2004). MMPs target a large diversity of substrates including growth factor receptors, cell adhesion molecules, chemokines, cytokines, apoptotic ligands, and pro-MMPs (Egeblad and Werb 2002, Overall and Kleifeld 2006, Cauwe et al. 2007). The characterization of new substrates as well as the generation of genetically modified animal models of gain or loss of MMP function has demonstrated the relevance of MMP activities in cancer development and progression (Folgueras et al. 2004). The gelatinase subgroup of MMPs, represented by MMP-2 (gelatinase A) and MMP-9

(gelatinase B), was the first class of MMP to be described as protumoral (Kleiner and Stetler-Stevenson, 1999, Duffy et al. 2000, Tester et al. 2004). Thereafter, their protumoral role has been extended to other MMPs (Pendas et al. 2004, Rio 2005, Overall and Kleifeld 2006). In this context, it is worth noting the key contribution of membrane-type MMP in cancer cell invasion. MT1-, MT2-, and MT3-MMP appear as a key triad for cancer cell invasion through the basement membrane (Hotary et al. 2002), while MT4-MMP is involved in metastatic dissemination of breast carcinomas (Chabottaux et al. 2006). Surprisingly, some MMPs display a protective function toward cancer progression (Matrisian and Lopez-Otin 2007). In fact, MMP-8-deficient mice challenged with carcinogens showed a markedly increased susceptibility to tumorigenesis in comparison to corresponding wild-type (WT) mice (Balbin et al. 2003). This study was the first report of an MMP having a protective role in cancer progression, validating MMP-8 as an antitarget in cancer therapy (Overall and Kleifeld 2006).

Key Determinants of the Tumor Compartment

In vivo, MMPs, in particular gelatinases and collagenases, have been found to be differentially regulated in premalignant and malignant skin SCC and breast carcinoma tumor cells and in their adjacent stroma (Borchers et al. 1997, Airola and Fusenig 2001, Werb et al. 1999). Yet, MMP expression data obtained in monolayer cultures of skin SCC cells did not identify a significant difference between benign and malignant tumor cells (Ala-Aho et al. 2000, Bachmeier et al. 2000) (Meade-Tollin et al. 1998). Secretion of a number of proteases such as proMMP-2, proMMP-9, and MMP-13 as well as very low levels of MMP-3 was already observed in monocultures of human immortalized nontumorigenic HaCaT cells (Papakonstantinou et al. 2005) and expression of MMP-3 and to a lesser extend MMP-9 increased with progression to benign (HaCaT-ras A-5) and even more so to enhanced malignant (HaCaT-ras II-4RT) tumor cells (Bachmeier and Nerlich 2002). However, when immortal and tumorigenic HaCaT cells were cultured in an in vivo-like environment on a collagen type-1 gel containing normal human dermal fibroblasts, a profound influence of the microenvironment, that is of the ECM and of stroma fibroblasts, on MMP expression became apparent. In these cocultures, immortal nontumorigenic HaCaT cells, benign (A-5) cells, and enhanced malignant (A-5RT3) cells exhibited a striking difference in their MMP expression pattern. In this tissue environment, MMP-1 mRNA and protein were strongly upregulated in malignant A-5RT3 cells, only weakly expressed in benign A-5 cells, and almost absent in immortalized HaCaT cells (Airola and Fusenig 2001). This enhanced expression of MMP-1 was further confirmed in two other malignant HaCaT-ras clones as well as in 2/2 primary squamous cell carcinoma lines. Finally, in vivo, malignant A-5RT3 tumors expressed MMP-1 mRNA consistently, preferentially at the tumor border. In contrast, MMP-1 expression was absent in the transplants of A-5 cells and HaCaT cells (Airola and Fusenig 2001).

This prominent difference in MMP-expression dependent on ECM and tissue organization suggests a very strong influence of the microenvironment with its stromal cells on the regulation of MMPs. The time course of malignant tumor growth that begins with an early onset of stromal activation, the rapid penetration of vessels and perivascular cells through the collagen gel toward the tumor cells, and their eventual infiltration into the malignant tumor tissue highlights another striking difference between benign and malignant transplants that lies in the differential dynamics of angiogenesis induction. Angiogenesis induction was transient in benign yet persistent in malignant tumors and found to be controlled by the regulation of the VEGF receptors 1 and 2. VEGFR-1 (vascular endothelial growth factor receptor) and -2 were downregulated in the stroma of benign, but continuously expressed in the malignant transplants. In contrast, VEGF-A expression persisted in both types of tumor cell transplants independently of the kinetics of angiogenesis (Skobe et al. 1997).

These observations gave a clear indication for the essential inductive or permissive role of the stroma for tumor invasion. In line with this essential role, VEGFR-2 blockade caused vessel regression and normalization as well as stromal maturation that ultimately resulted in a reversion from a highly malignant and invasive to a noninvasive tumor phenotype. Vessel regression was followed by downregulation of expression of both VEGFR-2 and VEGFR-1 on endothelial cells and increased association of α-smooth muscle actin-positive cells with small vessels indicating their normalization that was further supported by a regular ultrastructure. The phenotypic regression of an invasive carcinoma to a well-demarcated dysplastic squamous epithelium was accentuated by the establishment of a clearly structured epithelial basement membrane and the accumulation of collagen bundles in the stabilized connective tissue. This normalization of the tumor stroma border coincided with downregulated expression of the stromal MMP-9 and -13, which supposedly resulted in attenuated turnover of ECM components, permitting their structural organization (Fig. 17.2) (Vosseler et al. 2005). Thus, analysis of tumor–stroma interaction of skin SCCs in the matrix-inserted surface transplantation model provided abundant evidence for an essential role of the tumor stroma in regulating tumor malignancy as well as the expression of progression-associated MMPs. In particular, (1) a clear association of MMP expression in tumor and stromal cells with tumor progression was observed only in the tissue context of either an in vitro organotypic model or the transplantation model and (2) a stromal normalization achieved by VEGFR-2 blockade in highly malignant tumors induced a phenotypic reversion to a premalignant dysplasia that was in part mediated by a downregulation of stromal MMPs.

MMP-Deficient Mice as Models to Investigate the Stromal Contribution to Tumor Progression

To further elucidate the role of stromal MMPs in tumor progression and angiogenesis, the surface transplantation model has been recently applied to different MMP-deficient mice. The single deficiency of MMP-3, -8, and -11 or the combined

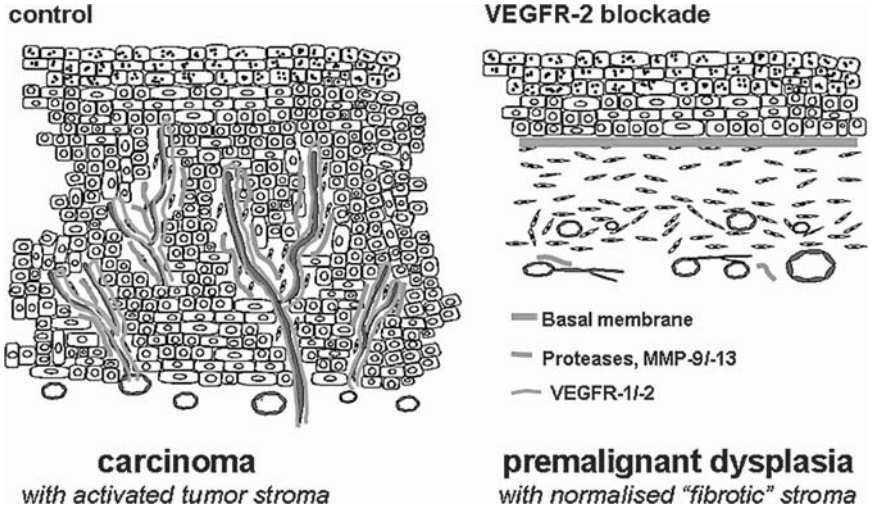

Fig. 17.2 Downregulation of stromal matrix metalloproteinase (*MMP*) expression by blockade of angiogenesis. Inhibiting angiogenesis in malignant transplants by the vascular endothelial growth factor receptor-2 (*VEGFR*-2) blocking antibody DC101 completely abrogates tumor vascularization and stromal MMP expression, and as a consequence, tumor invasion (Vosseler et al. 2005). (*See also* Color Insert I)

MMP-3/-9 deficiency did not affect tumor invasion and angiogenesis (Table 17.2) (Masson et al. 2005). Similarly, the absence of MMP-2 or MMP-9 in host tissue did not impair tumor progression (Table 17.2) (Masson et al. 2005). In sharp contrast, both tumor invasion and vascularization were impaired by the combined deficiency of MMP-2 and -9 (Masson et al. 2005). These results indicate that the concomitant stromal production of MMP-2 and -9 is required for tumor invasion and angiogenesis. Of particular importance is the necessity of specific interactions occurring between tumor cells and mesenchymal cells producing MMP-2, as well as inflammatory cells secreting MMP-9 (Masson et al. 2005). A synergistic contribution of MMP-2 and -9 in pathological angiogenesis has also been demonstrated in choroidal neoangiogenesis induced by laser burn (Lambert et al. 2003b). Interestingly and in contrast to most MMP deficiencies in mice described so far, the angiogenic response was accelerated and tumor invasion increased in MMP-19-deficient mice in comparison to WT mice (Jost et al. 2006). Indeed, endothelial cell recruitment was significantly increased 2 weeks after the transplantation, leading to an acceleration of tumor vascularization. As tumor vascularization precedes malignant invasion, this acceleration induced an early tumor invasion in MMP-19-deficient mice in comparison to corresponding WT mice, 21 days after transplantation. These data support the recent discovery of some MMPs as protective molecules toward cancer progression (Matrisian and Lopez-Otin 2007).

Table 17.2 Effects of MMP gene deletion on tumor angiogenesis and invasion

KO mice	MMP cellular sources		In vivo transplantations scoring		MMP effect on tumor	References
	Host cells	Epithelial tumor cells	Tumor invasion	Angiogenesis		
MMP-2	+	+	+++	+++	/	Masson et al. 2005
MMP-3	+	+	+++	+++	/	Masson et al. 2005
MMP-8	+	/	+++	+++	/	Unpublished data
MMP-9	+	/	+++	+++	/	Masson et al. 2005
MMP-11	+	/	+++	+++	/	Unpublished data
MMP-19	+	/	++++	++++	Negative regulator	Jost et al. 2006
MMP-2/-9	−	−	0	0	Positive regulator	Masson et al. 2005
MMP-3/-9	−	−	+++	+++	/	Masson et al. 2005

MMPs can be expressed by epithelial tumor cells (keratinocytes) or healthy stromal cells. Three weeks after tumor transplantation, angiogenesis and tumor invasion are determined by scoring + to +++ as described in the text and in Fig. 17.1. All corresponding wild-type (WT) mice presented an invasive and angiogenic score (+++).

Conclusion

Taken together all these data highlight several major aspects in protease expression in cancer. (1) Protease expression in tumor and stromal cells seems to contribute to tumor malignancy. Yet expression of tumor-derived proteases is clearly regulated by the in vivo tissue context, that is the tumor microenvironment. (2) Different proteases produced by stromal cells are the most important regulators of cancer development and progression. Yet, one has to keep in mind that the contribution of different stromal proteases has to be carefully evaluated especially in lieu of their potential usefulness as therapeutic targets since they sometimes act in an opposite manner. The matrix-inserted surface transplantation assay is therefore a highly valuable tool to identify the target (positive regulators) and anti-target (negative regulators) nature of different MMPs.

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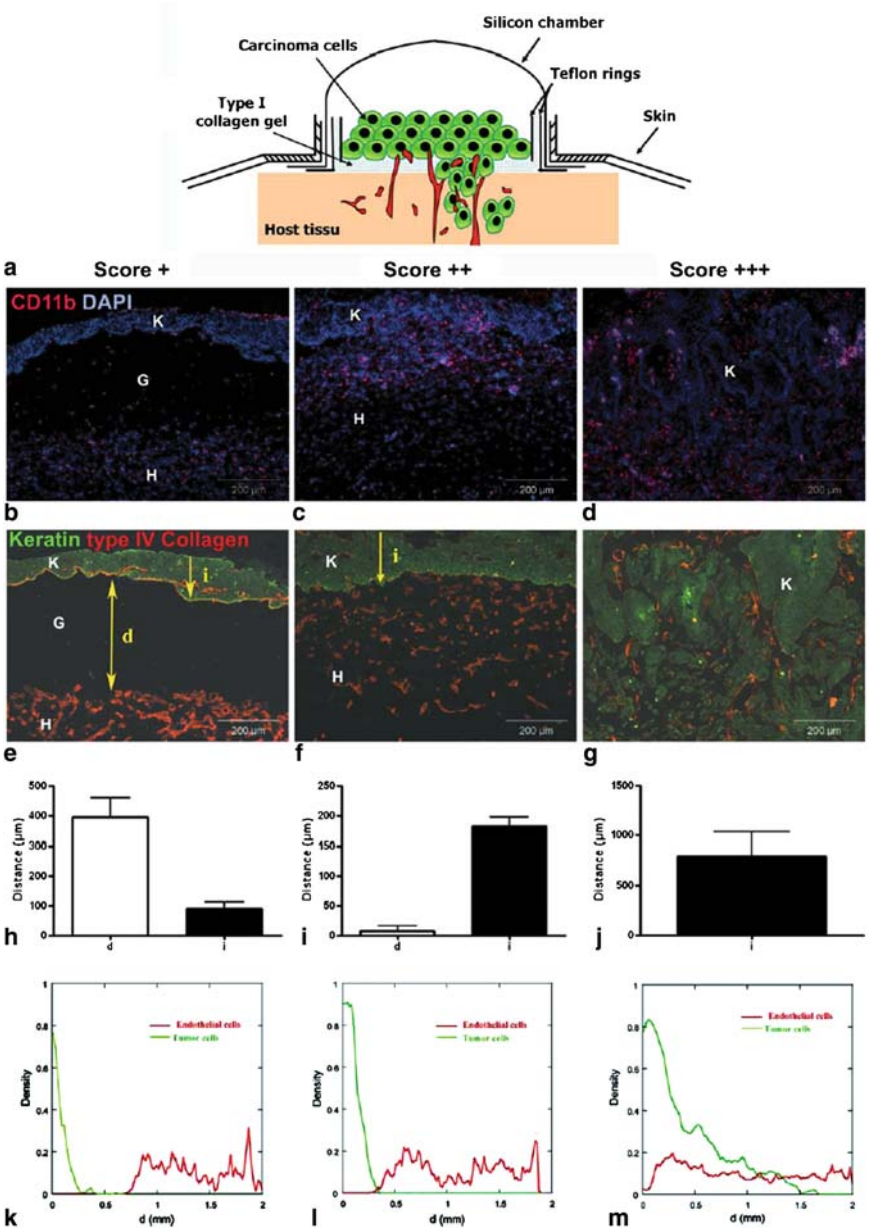

Fig. 17.1 Representation of skin carcinoma cell invasion in the surface transplantation model. **a** Schematic representation of the model: keratinocytes are cultured on the type-I collagen gel, mounted in concentric teflon rings and covered with a hat-shaped silicone chamber. **b–g** Different stages of tumor invasion (score +, ++, +++) are observed after 1 (**b, e**), 2 (**c, f**), or 3 (**d, g**) weeks of transplantation. After 1 week of transplantation, tumor cells proliferate to form a multilayered epithelium, and few inflammatory cells infiltrate the collagen gel (**b**). The collagen gel is

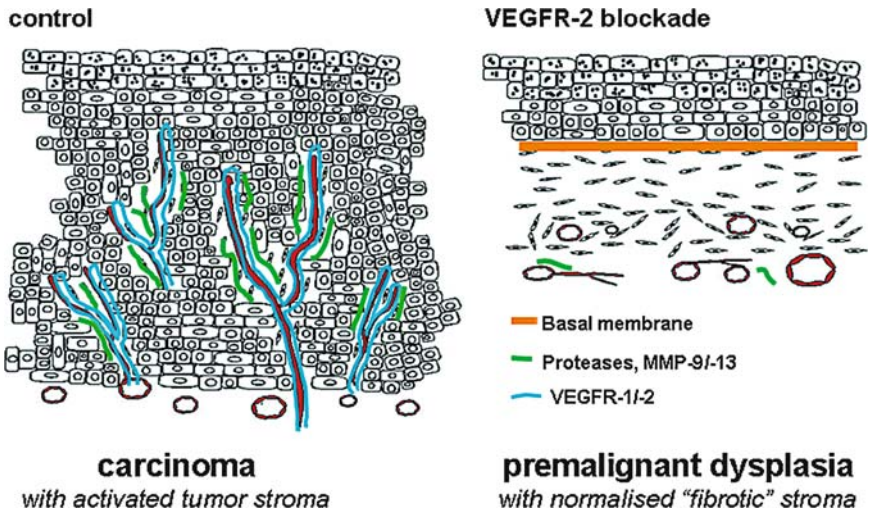

Fig. 17.2 Downregulation of stromal matrix metalloproteinase (*MMP*) expression by blockade of angiogenesis. Inhibiting angiogenesis in malignant transplants by the vascular endothelial growth factor receptor-2 (*VEGFR*-2) blocking antibody DC101 completely abrogates tumor vascularization and stromal MMP expression, as a consequence, tumor invasion (Vosseler et al. 2005).

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progressively replaced by a granulation tissue at 2 weeks (**c**), leading to a vascularized tumor at 3 weeks (**d**). **b–d**: Immunostaining of CD11b, a marker of granulocytes and monocytes/macrophages. **e–g** Immunostaining of keratinocytes (green) and vessels (red). **e** A score of + is attributed when blood vessels remain below the collagen gel or particularly the matrix. **f** When blood vessels get into close contact with malignant epithelial layer, a score of ++ is assigned. **g** The score is +++ when tumor and blood vessels are intermingled. **h–j** Tumor invasion can be quantified by manual measurements of the distance "*i*," between the top of tumor cell layer and the deepest front of tumor spread (yellow arrows) (**e, f**). In this system, vascularization is estimated by measuring the distance "*d*" separating tumor cells from the front blood vessel migration (**e**). Quantification by computerized image analysis consists in determining the distribution of tumor/endothelial cell density as a function of the distance to the upper boundary of tumor (**k–m**).