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Matrix metalloproteinases in keratinocyte carcinomas

Pilvi Riihilä[1,2] | Liisa Nissinen[1,2] | Veli-Matti Kähäri[1,2]

[1]Department of Dermatology, University of Turku and Turku University Hospital, Turku, Finland

[2]FICAN West Cancer Centre Research Laboratory, University of Turku and Turku University Hospital, Turku, Finland

Correspondence
Veli-Matti Kähäri, Department of Dermatology, University of Turku and Turku University Hospital, Hämeentie 11 TE6, FI-20520 Turku, Finland.
Email: veli-matti.kahari@utu.fi

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Abstract

The incidence of cutaneous keratinocyte-derived cancers is increasing globally. Basal cell carcinoma (BCC) is the most common malignancy worldwide, and cutaneous squamous cell carcinoma (cSCC) is the most common metastatic skin cancer. BCC can be classified into subtypes based on the histology, and these subtypes are classified further into low- and high-risk tumors. There is an increasing need to identify new therapeutic strategies for the treatment of unresectable and metastatic cSCC, and for aggressive BCC variants such as infiltrating, basosquamous or morpheaform BCCs. The most important risk factor for BCC and cSCC is solar UV radiation, which causes genetic and epigenetic alterations in keratinocytes. Similar gene mutations are noted already in sun-exposed normal skin emphasizing the role of the alterations in the tumor microenvironment in the progression of cSCC. Early events in cSCC progression are alterations in the composition of basement membrane and dermal extracellular matrix induced by influx of microbes, inflammatory cells and activated stromal fibroblasts. Activated fibroblasts promote inflammation and produce growth factors and proteolytic enzymes, including matrix metalloproteinases (MMPs). Transforming growth factor-β produced by tumor cells and fibroblasts induces the expression of MMPs by cSCC cells and promotes their invasion. Fibroblast-derived keratinocyte growth factor suppresses the malignant phenotype of cSCC cells by inhibiting the expression of several MMPs. These findings emphasize the importance of interplay of tumor and stromal cells in the progression of cSCC and BCC and suggest tumor microenvironment as a therapeutic target in cSCC and aggressive subtypes of BCC.

KEYWORDS

basal cell carcinoma, extracellular matrix, fibroblasts, keratinocytes, matrix metalloproteinase, squamous cell carcinoma

1 | INTRODUCTION

Cutaneous malignancies are the most common cancers worldwide, and their incidence is increasing globally causing mortality in ageing population. Non-melanoma skin cancers (NMSC) are classified to keratinocyte-derived basal cell carcinoma (BCC), and cutaneous squamous cell carcinoma (cSCC), which are the most common cutaneous malignancies and more than a third of patients develop multiple tumors.[1–3] Other types of non-melanoma skin cancer, including Merkel cell carcinoma, adnexal tumors and sarcomas, are less common and differ in their cell type, behaviour and epidemiology from keratinocyte carcinomas (KC).[1] Therefore, KC has become the

Pilvi Riihilä and Liisa Nissinen equal contributions to this work.

preferred term for BCC and cSCC, both of which are derived from epidermal keratinocytes.[1]

The most important risk factors for cSCC are long-term exposure to solar ultraviolet (UV) radiation, immunosuppression, chronic inflammation, chronic cutaneous ulceration and human papilloma virus (HPV) infection.[4,5] Approximately 2%-4% of primary cSCCs metastasize, and this is the main cause of mortality of NMSC.[3,6] The prognosis of patients with metastasic cSCC is poor, emphasizing the need for new therapeutic strategies and for biomarkers to predict the risk of aggressive behaviour of primary cSCC.[3,7,8]

The mutation rate of cSCC is one of highest among the malignant tumors, and the majority of mutations found in cSCC are UV-induced, known as "UV-signature."[9] The important early event in cSCC development is mutation and inactivation of tumor suppressor function of the tumor protein 53 (TP53), which in turn leads to further accumulation of simple mutations including the loss-of-function mutation of *NOTCH1*.[10] Inactivation of p53 also results in downregulation of NOTCH1 expression.[11] Furthermore, driver mutations in different genes, including *NOTCH1*, *NOTCH2*, *EGFR*, *HRAS*, *KRAS* and *PIK3CA*, have been identified in cSCC.[12–14] Additional alterations, for example in non-coding RNAs, are obviously required for the progression of premalignant lesion, actinic keratosis (AK), to cSCC in situ (cSCCIS), and finally to invasive and metastatic cSCC, since keratinocytes in chronically sun-exposed normal skin also harbour several of these driver mutations with high density.[15,16]

The influx of microbes and inflammatory cells into tumor microenvironment results in activation of proteolytic remodelling and alteration in the composition of epidermal basement membrane and dermal extracellular matrix (ECM) (Figure 1). The remodelling of ECM and tumor microenvironment paves way for tumor progression from premalignant forms to invasive and metastatic cSCC.[17–19] An early sign of ECM remodelling in cSCC progression is the loss of collagen XV and XVIII from the basement membrane in AK, while the stroma of AK lesions or normal dermal keratinocytes remain negative at this stage.[17] The remodelling of these matrix molecules continues during the progression of cSCC, and collagen XV and XVIII appear later in the stroma of cSCC tumors.[17] In addition, collagen VII, a component of anchoring fibrils, regulates signalling of transforming growth factor-β (TGF-β) and decreases the secretion of vascular endothelial growth factor (VEGF), and this way suppresses angiogenesis (Figure 2).[18] In recessive dystrophic epidermolysis bullosa (RDEB), collagen VII is absent and this promotes the aggressive progression of cSCC in RDEB patients.[20]

The remodelling of ECM and basement membrane is mediated by extracellular proteinases, especially matrix metalloproteinases (MMPs), which digest fibrillar collagens, gelatin, elastin, proteoglycans and fibronectin.[21] MMPs are produced by tumor cells,

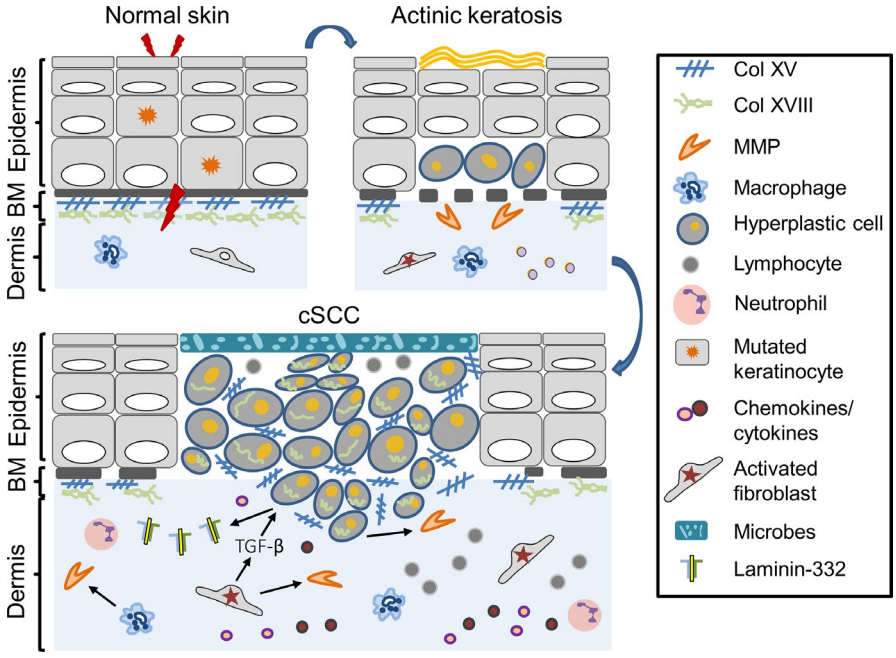

FIGURE 1 Cellular interactions in cSCC tumor microenvironment. A well-organized, intact basement membrane (BM) separates epidermis from the dermal layer. Environmental insults, such as UV radiation, target cells both in epidermal and dermal layer. This leads to genetic and epigenetic changes in dermal fibroblasts and keratinocytes. Already in chronically sun-exposed normal skin, epidermal keratinocytes harbour mutations in the driver genes of cSCC progression. Differentiation of keratinocytes is disturbed during transition of normal skin to premalignant actinic keratosis (AK), and this leads to neoplastic epithelium containing atypic hyperplastic cells. Production of matrix metalloproteinases (MMPs) by activated stromal fibroblasts, macrophages and neoplastic cells is induced in AK. In AK, collagen XV and XVIII are lost in the BM and influx of inflammatory cells takes place. The number of inflammatory cells increases during later stage of cSCC progression. Activated fibroblasts produce MMPs, growth factors and promote inflammation, growth and invasion of tumor cells. In addition, activated stromal fibroblasts produce transforming growth factor-β (TGF-β) and induce production of laminin-332 by Ras-transformed keratinocytes and promote invasion of these transformed epithelial cells

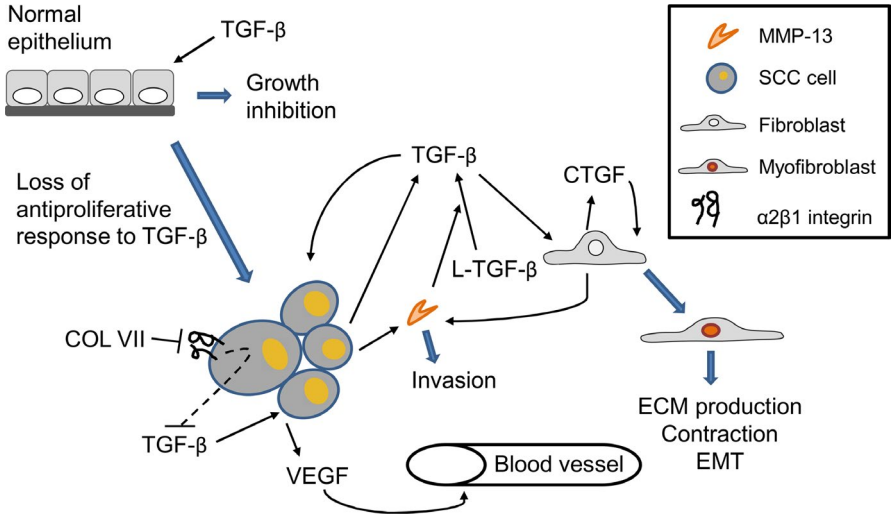

FIGURE 2 Role of transforming growth factor-β (TGF-β) in cSCC progression. TGF-β shows antiproliferative effect on the cells in normal epithelium, but the epithelial cells in developing tumors start to produce TGF-β and become refractory to the growth suppressive effect of TGF-β. Production of TGF-β by SCC cells induces the expression of MMP-13 in autocrine manner. MMP-13 activates latent TGF-β (L-TGF-β) from the ECM and generates a positive feedback loop. MMP-13 also promotes invasion of cSCC cells and survival of SCC cells and fibroblasts. TGF-β induces stromal fibroblasts to produce connective tissue growth factor (CTGF), which stimulates fibroblast proliferation. Fibroblasts undergo phenotypic change to myofibroblasts, which show increased production of ECM components, induce contraction and epithelial-to-mesenchymal transition (EMT). Blockage of the interaction between Col VII and α2β1 integrin increases activation of TGF-β. TGF-β signalling increases vascular endothelial growth factor (VEGF) secretion and endothelial cell tube formation

surrounding stromal fibroblasts, and by tumor-associated inflammatory cells.[22] MMPs also have important roles in normal tissue remodelling during development and wound healing.[22] MMPs are important mediators of invasion and metastasis, and they also regulate the activity of growth factors, cytokines, chemokines and their cell surface receptors and this way promote cancer progression and inflammation in the tumor microenvironment.[23]

2 | KERATINOCYTE CARCINOMAS

Keratinocyte-derived NMSCs, *that is* KCs, are the most common skin cancers among caucasian population. BCC is the most common human malignancy, but cSCC is responsible for the majority of deaths of patients with KCs.[25,26] Of KCs, cSCC is the most common metastatic skin cancer and it comprises about 20% of KCs.[3,24] AK is premalignant form of cSCC, which can develop to in situ cSCC (cSCC-CIS, Bowen's disease) and finally to invasive and potentially metastatic cSCC. The prognosis of metastatic cSCC is poor with current treatments, and the need for targeted therapies is evident.[2–4,6,25,26] The main risk factors for cSCC are cumulative exposure to solar UV radiation, immunosuppression, chronic cutaneous ulceration and chronic inflammation of the skin.[1,27–29]

Although BCCs and cSCCs harbour a high burden of UV-induced mutations, they do not share many genetic alterations, except inactivation of tumor suppressor p53.[1] In BCC, loss of PTCH1 receptor function results in activation of the G protein-coupled receptor SMO and constitutive activation of the Hedgehog signalling pathway.[1]

The mutational burden of cSCC is one of the highest detected in any type of cancer comprising over 1700 mutations in the primary cSCC tumor exomes and on average 50 mutations/mega base pair DNA.[10,30,31] The most important risk factor, UV radiation, induces mutation and functional inactivation of the tumor suppressor, the tumor protein 53 gene (*TP53*) in epidermal keratinocytes as an early event in keratinocyte carcinogenesis. p53 maintains genomic stability, and its inactivation leads to marked accumulation of UV-induced simple mutations known as "UV-signature" showing mutation pattern C > T and CC > TT predominance in cSCC (COSMIC signature 7).[30,32] Accordingly, *TP53* mutation can be detected in 90% of cSCCs.[9,33] Mutation and inactivation of one *TP53* allele leads to apoptosis of keratinocytes by UV radiation. Inactivation of both *TP53* alleles renders keratinocytes resistant to apoptosis allowing further accumulation of UV-induced mutations and eventually progression to cSCC.[12,16,34,35,36]

Loss-of-function mutations of *NOTCH1* and *NOTCH2* are also early events in the development of cSCC detected in up to 85% of cSCCs, demonstrating the tumor suppressor function of Notch signalling pathway in keratinocytes.[10,30]

High-level amplification of epidermal growth factor receptor (EGFR) or mutational activation *EGFR*, *HRAS* and *KRAS* underline the role of extracellular signal regulated kinase 1/2 (ERK1/2) signalling and EGFR in the progression of cSCC.[37,38] Moreover, EGFR signalling downregulates the expression of p53 and NOTCH1.[14,39] Additional driver gene mutations noted in cSCC comprise activation of *PIK3CA* indicating the role of phosphatidyl inositol 3-kinase (PI3K-AKT) signalling in the progression of cSCC.[12,14,37,40]

Additional signalling molecules involved in the development of cSCC are gene mutations in signal transducer and activator of transcription 3 (STAT3), p63-fibroblast growth factor receptor-2 (p63-FGFR2) axis and Wnt/β-catenin.[37,40] Similar driver gene

mutations have been identified even in chronically sun-exposed normal epidermal keratinocytes in chronically sun-exposed normal looking skin indicating that other alterations, for example in non-coding RNAs and in the microenvironment of premalignant lesions, are required in the keratinocyte carcinogenesis towards invasive cSCC.[15,41,42]

Immunosuppression and chronic inflammation are important risk factors for the progression of cSCC. UV radiation has an effect on T-cell subtypes in skin resulting to inflammation and immunosuppression.[43] Immunosuppressive medication, for example in organ transplant recipients (OTRs), is associated with significantly increased risk of developing cSCC.[1,44,45] In addition, cSCC is more common than BCC in immunosuppressed individuals.[5,46] cSCCs in OTRs possess a higher rate of recurrence, metastasis and mortality.[47–51] The UV-induced "UV-signature" mutations are also noted at the early stage of cSCC carcinogenesis in immunosuppressed individuals as in immunocompetent individuals, but the mutation frequency is higher and the level of chromosomal instability is increased compared to tumors in immunocompetent individuals.[52] Especially immunosuppressive medication with azathioprine is associated with specific mutation signature in cSCC tumors indicating that this drug can promote cSCC progression by causing widespread DNA damage and protein oxidation leading to increased UVB mutagenicity.[30]

Infection with human papilloma virus (HPV) and loss of immune surveillance, are risk factors of cSCC especially in immunosuppressed patients. β-HPV has been suggested as a marker for the risk of cSCC development in OTRs.[53] Activation of inflammatory signalling pathways appears to play a role in cSCC progression in immunosuppressed patients. Tumor necrosis factor-α (TNF-α) signalling pathway activation has been detected in AK lesions in immunosuppressed OTRs, and immunosuppressive medication has been proposed to promote the progression of cSCC by increasing the level of TGF-β and VEGF in cSCCs.[51,54]

3 | MATRIX METALLOPROTEINASES

MMPs are a ubiquitously expressed diverse group of zinc-dependent proteolytic enzymes with wide substrate specificity and multiple physiological functions. MMPs belong to metzincin superfamily, which is characterized by the presence of a highly conserved motif containing three histidine residues, which chelate a zinc ion (Zn^{2+}) in the catalytic site.[55] The expression of MMPs is regulated at transcriptional level. MMPs are produced either as soluble or cell surface-anchored endopeptidases, which cleave ECM components and also several non-matrix substrates including growth factors, chemokines, cytokines, growth factor receptors and cell surface adhesion receptors.[56] MMPs show marked differences in their tissue specific expression and substrate specificity. In addition, expression and activity of various MMPs has been reported in pathological conditions, such as inflammatory diseases and cancer.[56–62]

Altogether, 23 human MMPs have been identified. They are usually classified to different subgroups based on their structure, substrate specificity and function. Most MMPs show homologous structure containing four distinct functional domains: signal peptide, propeptide, catalytic domain and hemopexin-like domain. The N-terminal signal peptide is responsible for secretion of these proteases. MMPs contain a highly homologous catalytic domain, which is required for the proteolytic activity, and a propeptide, which regulates MMP activity by interacting with the Zn^{2+} ion in catalytic pocket through a conserved cysteine residue. This covalent interaction, "cysteine switch," retains the enzyme in the latent, catalytically inactive state.[63] MMP-1, -8 and -13 are included in subgroup of *collagenases*. Other subgroups are *gelatinases* (MMP-2 and -9), *stromelysins* (MMP-3 and -10), *matrilysins* (MMP-7 and -26), *stromelysin-like* MMPs (MMP-11 and -12), *transmembrane* MMPs (MMP-14, -15, -16 and -24), *glycosyl-phosphatidyl-inositol (GPI)-type* MMPs (MMP-17 and -25), *MMP-19-like* MMPs (MMP-19 and -28) and *other* MMPs (MMP-20, -21, and -23A, -23B).[58–60] Most MMPs are secreted as inactive zymogens and then activated in the extracellular space. ProMMPs may be activated by several proteinases, for example trypsin, plasmin, kallikrein, mast cell tryptase and other MMPs.[55] The activity of MMPs is regulated by non-specific protease inhibitors, for example α1-antiprotease and α2-macroglobulin and by specific inhibitors, tissue inhibitors of metalloproteinases (TIMPs).[64]

As mentioned above, development of cSCC involves accumulation of substantial mutational burden, mainly due to cumulative UV exposure. In addition, UVB irradiation has been noted to influence the gene transcription through histone acetylation in immortalized keratinocytes.[65] Interestingly, UVB has been shown to activate the transcription of genes in locus 11q22.3, which contains a cluster of several MMP genes.[66] Among them are cSCC progression related genes *MMP13*, *MMP12*, *MMP3*, *MMP1* and *MMP10* regulated by STAT3 (Figure 3A).[67] Concurrent induction of the expression of these MMPs results in potent local proteolytic activity achieved by activation of latent MMPs by other MMPs (Figure 3B). For example, MT1-MMP on the surface of tumor and stromal cells can activate proMMP-13 either directly or indirectly by activating proMMP-2. In addition, MMP-3, MMP-10 and MT2-MMP can activate proMMP-13,[68,69] and proMMP-2 and proMMP-9 can in turn be activated by MMP-13.[70]

4 | MMPs IN KERATINOCYTE CARCINOMAS

Proteolytic remodelling and cleaving of stromal ECM plays an important part in cancer progression. Tumor cells as well as stromal and inflammatory cells in tumor microenvironment serve as source of several proteinases and their inhibitors, including plasminogen activators and their inhibitors, serine proteinase inhibitors (serpins), MMPs, a disintegrin-like and metalloproteinases domain (ADAMs) and ADAMs with thrombospondin type 1 motif (ADAM-TSs), and TIMPs involved in this process.[21] The expression of several MMPs

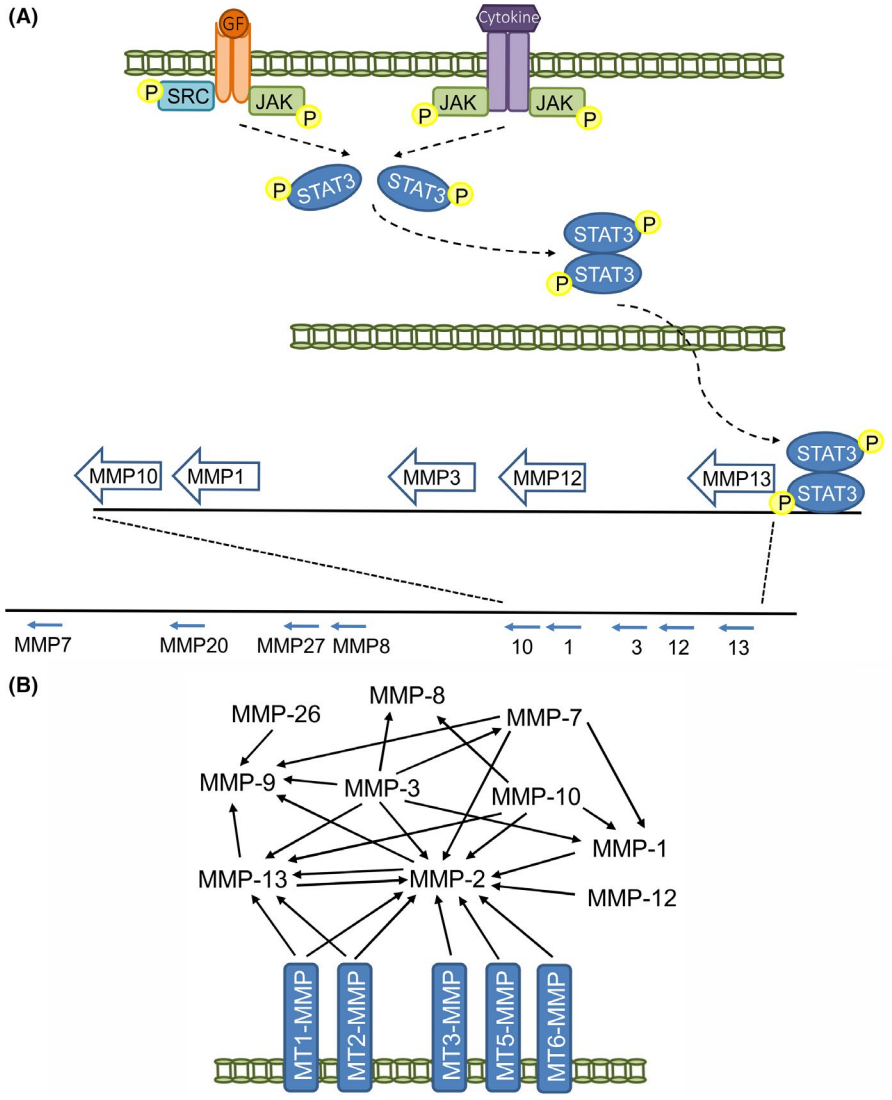

FIGURE 3 MMP gene cluster and MMP activation network. A, MMP gene cluster at chromosome 11q22.3. Arrows show the corresponding genes and the orientation of transcription. STAT3 signalling is induced by cytokines and growth factors. This leads to STAT3 phosphorylation and translocation to nucleus where it induces the transcription of MMP genes in the cluster. B, MMPs can activate each other in the pericellular space, for example in tumor microenvironment. Arrows indicate the direction of the activation. MT1-MMP (MMP-14), MT2-MMP (MMP-15), MT3-MMP (MMP-16), MT5-MMP (MMP-24), MT6-MMP (MMP-25)

either by tumor cells or stromal fibroblasts has been reported in KCs, cSCC and BCC (Tables 1 and 2).

4.1 | MMPs in cutaneous squamous cell carcinoma

The expression of MMPs in tumor microenvironment is associated with cancer progression and metastasis.[71,72] The regulation of cancer progression is mediated by different MMP functions such as cleavage of ECM, cytokines, chemokines and growth factors, regulating their availability and activity in the tumor microenvironment.[56,73] The expression of several MMPs by tumor cells, stromal fibroblasts or inflammatory cells has been reported at different stages of the development of cSCC tumors (Table 1).

MMP-1 (collagenase-1) is secreted by tumor cells or stromal fibroblasts in cSCC and has a role in tumor angiogenesis.[74] In addition, upregulation of MMP-1 expression is detected in AKs indicating its role in early cSCC progression.[75] MMP-7 (matrilysin-1), MMP-9 (gelatinase B) and MMP-14 (MT1-MMP) are expressed by cSCC cells, but the expression is absent or lower in normal keratinocytes, AKs or cSCCISs.[75–78] MMP-7 expression is associated with the aggressive behaviour of cSCC tumors. In aggressive form of cSCC, RDEB-associated cSCC (RDEBSCC), tumor cells express MMP-7 and the staining intensity of MMP-7 was significantly stronger in RDEBSCCs compared to sporadic cSCC.[79] In addition, MMP-7 promotes proliferation of cSCC cells by cleaving and activating heparin binding epidermal growth factor (HB-EGF) on the surface of cSCC cells.[80] MMP-7 is detected at the invasive edge of cSCC and promotes cSCC cell migration.[77,81] MMP-9 and MMP-2 are upregulated in cSCC tumors,[82,83] and higher MMP-2 and MMP-9 expression is noted in the invasive margin of cSCC than in peritumoral areas.[84] Furthermore, MMP-2 is associated with the histologic grade of cSCC tumor and lower expression of MMP-2 has been noted in AK and SCCIS than in cSCC.[85] MMP-2 expression is detected also in the peritumoral epidermal layer in cSCC suggesting association with UV-induced damage.[86]

MMP-9 expression is also noted in inflammatory cells in the stromal compartment of cSCC.[77,79] Accordingly, MMP-9 and MMP-11 (stromelysin-3) colocalize with $CD163^+$ tumor-associated macrophages (TAM) in cSCC indicating that these MMPs are associated with infiltration of TAMs in cSCC.[87,88] In addition, MMP-8

TABLE 1 MMPs and TIMPs in cutaneous squamous cell carcinoma (cSCC), in recessive dystrophic epidermolysis bullosa-associated cSCC (RDEBSCC), cSCC in situ (cSCCIS), actinic keratosis (AK) and normal skin

	Expression		
MMP	**Present**	**Absent**	**Reference**
MMP-1 (collagenase-1)	cSCC tumor cells		[74]
	Stromal fibroblasts in cSCC		[74]
	AK		[75]
MMP-2 (gelatinases A)	cSCC tumor cells, in invasive edge		[82] [83]
	Low expression in AK and cSCCIS		[85]
MMP-13 (collagenase-3)	Tumor cells, stromal fibroblasts in cSCC Tumor cells in cSCCIS Tumor cells in RDEBSCC	AK, normal skin	[75] [76] [79]
MMP-7 (matrilysin-1)	cSCC tumor cells, in invasive edge, CSCCIS	normal skin	[79] [77]
	RDEBSCC tumor cells	AK and keratinocytes in normal skin	[79]
MMP-9 (gelatinase B)	cSCC tumor cells, in invasive edge		[82] [77] [83]
	Inflammatory cells in cSCC stroma		[79] [77]
	TAMs in cSCC stroma		[87] [88]
MMP-10 (stromelysin-2)	Laminin-5 positive cSCC cells	Premalignant lesions of cSCC	[89]
	cSCC tumor cells		[90]
	Inflammatory cells in cSCC stroma		[90] [77]
MMP-11 (stromelysin-3)	TAMs in cSCC stroma		[87]
MMP-14 (MT1-MMP)	cSCC tumor cells, in invasive edge		[92]
	Stromal fibroblasts in cSCC		[89]
MMP-12 (macrophage metalloelastase)	cSCC tumor cells		[91]
MMP-3 (stromelysin-1)	Stromal cells in cSCC		[75]
MMP-19	Hyperproliferative epidermal keratinocytes	Invasive cSCC	[95]
	Fibroblasts		[152]
MMP-21	cSCC tumor cells	Hyperproliferative keratinocytes	[98]
MMP-26 (matrilysin-2)	Well differentiated cSCC tumor cells	Poorly differentiated cSCC	[97]
MMP-28 (epilysin)		Invasive cSCC	[96]
TIMP-1	Stromal fibroblasts of cSCC		[84]
TIMP-2	Stromal fibroblasts of cSCC		[153] [154] [84]
TIMP-3	cSCC stroma		[100]

(neutrophil collagenase or collagenase 2) and MMP-10 are expressed in peritumoral inflammatory cells in cSCC.[77,89,90] MMP-10 is expressed by tumor cells in cSCC, especially in tumors with prominent inflammation.[89,90] Furthermore, MMP-10 expression in cSCC tumor cells is stronger in poorly differentiated (grade II and III) tumors than in well differentiated (grade I), cSCCs.[89,90] Expression of MMP-3 is detected both in tumor and stromal cells in cSCCs, but it does not colocalize with MMP-10.[89] MMP-12 (macrophage metalloelastase) is also expressed by tumor cells in cSCCs, but not in premalignant tumors AK or cSCCIS, or in normal keratinocytes.[91]

The expression MMP-14 and MMP-2 correlates with the invasive capacity of cSCC cell lines, and MMP-14 is detected in the invasive

TABLE 2 MMPs and TIMPs in basal cell carcinoma (BCC)

MMP	Expression	Reference
MMP-1 (collagenase-1)	BCC tumor cells in invasive edge, stromal fibroblasts, macrophages, inflammatory cells, endothelial cells	[102] [103] [123]
MMP-2 (gelatinase A)	BCC stromal fibroblasts, inflammatory cells, vascular endothelial cells	[78] [84] [105] [106] [107]
MMP-13 (collagenase-3)	BCC cells in invading edge, fibroblasts, inflammatory cells, vascular endothelial cells	[75] [119] [121] [123]
MMP-7 (matrilysin-1)	BCC tumor cells	[112]
MMP-9 (gelatinase B)	BCC tumor cells, stromal fibroblasts in invasive area, inflammatory cells, endothelial cells	[84] [109] [102] [106] [123]
MMP-10 (stromelysin-2)	BCC epithelium, tumor stroma, endothelium and macrophages	[90] [102]
MMP-11 (stromelysin-3)	BCC stromal cells	[116]
MMP-14 (MT1-MMP)	BCC tumor cells in invasive edge, stromal fibroblasts	[89] [122] [78]
MMP-12 (macrophage metalloelastase)	TAMs in BCC stroma	[91]
MMP-3 (stromelysin-1)	BCC stromal cells	[111]
MMP-21	BCC tumor cells	[98]
TIMP-1	BCC cells in invading edge, fibroblasts, inflammatory cells, vascular endothelial cells	[123]
TIMP-2	BCC stromal cells	[84]

edge of cSCC suggesting a role in cSCC aggressiveness and invasion.[89,92] MMP-14 is also expressed abundantly by stromal fibroblasts in cSCC.[89]

MMP-13 (collagenase-3) expression is noted in cSCC tumor cells and stromal fibroblasts and in tumor cells in a subset of cSCCIS, but not in normal skin or AKs.[75,76,79] MMP-13 promotes growth and invasion cSCC *in vivo*, and the expression is noted in the invasive edge of cSCC.[75,76] In addition, the expression of MMP-13 is prominent in large and locally invasive head and neck SCCs and the expression is associated with poor prognosis.[76,93] MMP-13 expression correlates with epithelial-to-mesenchymal transition (EMT) indicating that MMP-13 promotes cell migration.[74] Moreover, the expression of MMP-7, MMP-12 and MMP-13 is noted in cSCCs, which have developed in chronic ulcers.[94] In contrast, the expression of MMP-19 is absent in the invasive parts of cSCC in chronic ulcers and in UV-induced cSCCs.[94,95] These observations support hypothesis that the expression profile of MMPs in the tumor microenvironment is similar in SCCs in chronic ulcers and UV-induced cSCCs.

MMP-28 (Epilysin) is expressed by keratinocytes in normal skin and wound repair, but it is not expressed in cSCC regardless of the level of differentiation.[96] Expression of MMP-26 is detected in tumor cells of well and moderately differentiated cSCCs, but not in poorly differentiated (grade III) tumors.[97] MMP-26-positive atypical keratinocytes can also be detected in premalignant lesions, suggesting a role for MMP-26 early in cSCC development. Expression of MMP-21 is not detected in normal skin or in skin conditions characterized by epidermal hyperproliferation, such as psoriasis and chronic wounds.[98] MMP-21 protein is not expressed in cSCCIS, but it is expressed in cSCCs in tumor cells in the invasive front, particularly in poorly differentiated tumors.[98,99] These results suggest that MMP-21 is specifically induced at the invasive stage of cSCC development. In addition, MMP-21 is expressed by stromal fibroblasts in cSCCs.[99] Expression of TIMP-3 mRNA is detected in stromal cells in squamous cell carcinomas.[100]

4.2 | MMPs in basal cell carcinoma

The expression of several MMPs and TIMPs by tumor cells or stromal fibroblasts has been reported in BCC (Table 2).[71] Distinct MMPs are expressed in different types of BCCs indicating that they may have an effect on the histopathologic type of BCC and serve as markers for more aggressive BCC subtype.

The expression level of MMP-1 at mRNA and protein is elevated in BCC tumors compared to unaffected tissue.[101] MMP-1 is expressed by stromal fibroblasts, macrophages and in BCC tumor margins, and the expression is more abundant in superficial BCC than in nodular BCC.[102,103] Moreover, MMP-1 expression is stronger in invasive morpheaform and recurrent BCC than in superficial, micronodular and cystic BCC, suggesting a role for MMP-1 in ECM remodelling and tumor invasion in BCC.[103] Similarly, stromal expression of MMP-1 is associated with loss of palisading arrangement in peripheral cells of BCC nests, which is considered as a marker for poor differentiation.[104]

MMP-2 is expressed in stromal fibroblasts, inflammatory cells and vascular endothelial cells.[102,103,105–107] Interestingly, the expression of MMP-2 by fibroblasts was downregulated by interaction with BCC cells in co-culture of fibroblasts and BCC cells.[108] In addition to tumor stroma, MMP-2 is detected also in tumor cells in BCC.[109] MMP-2 expression is lower in BCC stroma than in cSCC.[78,82] No difference in MMP-2 expression has been noted between different subtypes of BCC.[110]

Expression of MMP-3 at mRNA and protein level is elevated in BCC tumors compared to unaffected tissue.[89,111] MMP-3 is expressed mainly by stromal fibroblasts and infrequently in sclerosing cancer islands.[89,111] Expression of MMP-7 appears to be associated with aggressive invasive BCCs. MMP-7 is only noted in the tumor-stromal interface of recurrent or morpheaform/infiltrative BCC.[112] The expression of MMP-8 mRNA is significantly elevated in BCC compared to unaffected tissue. However, no significant increase in the protein levels of MMP-8 could be detected.[101]

MMP-9 expression is detected in stromal fibroblasts in the invasive area of infiltrating BCC[84,106,109] and the mRNA and protein expression level of MMP-9 is elevated in BCC compared to unaffected tissue.[101] MMP-9 is also detected in the BCC tumor epithelium and is associated with inflammatory cell infiltrate in the microenvironment of BCC.[84,109] MMP-9 is expressed differently in BCC subtypes, and IHC staining of MMP-9 is more positive in superficial BCC tumor subtype.[102] In addition, higher MMP-9 expression was noted in infiltrative BCC than in nodular BCC.[110] However, MMP-9 expression does not correlate with recurrence of BCC or with clinical factors.[113] MMP-9 expression in cSCC stroma is stronger than in BCC.[82,114] In organ culture model of BCC, epithelial cells were mainly responsible for MMP-9 expression and stromal cells for the expression of MMP-1.[115]

MMP-10 is detected in the BCC epithelium, tumor stroma, endothelium and macrophages.[89,102] Strong expression of MMP-10 is noted in nodular and aggressive BCC subtypes such as invasive or fibrosing BCC tumors.[89,102] In these invasive types of BCC, MMP-10 was detected locally close to the necrotic areas on the surface of BCC. MMP10 expression is more frequent in cSCC tumors than in BCC.[89] These findings suggest that MMP-10 expression could serve as marker for invasive behaviour of BCC. MMP-12 is detected in macrophages of invasive fibrosing BCC more often than in less aggressive BCC types keratotic or adenoid BCCs suggesting a role for MMP-12 in the invasive capacity of BCC.[91] MMP-11 is also detected in the stromal cells of BCC, especially in aggressive forms BCC.[116,117] Furthermore, MMP-11 mRNA is overexpressed in tumor tissue of BCC, and the expression is lower than in cSCCs.[118]

MMP-13 mRNA is expressed in focal areas of keratinized cells in BCC, suggesting a potential role in terminal differentiation of these epithelial cells.[75] In addition, strong IHC staining for MMP-13 was detected in vascular endothelial cells in the BCC microenvironment suggesting a role for MMP-13 in angiogenesis in BCC.[119] Moreover, MMP-13 is associated with invasion potential of BCC.[120] Expression of MMP-13 is detected in the invading edge of BCC tumor, in fibroblasts, inflammatory cells and endothelial cells. Additionally, the aggressive subtypes of BCC (infiltrative-morpheaform, metatypical and micronodular) stained more strongly for MMP-13, especially in metatypical BCC in the areas of squamous cell differentiation.[121]

MMP-14 is expressed in the invasive edge of BCC in high-risk BCCs, (mixed, infiltrative, morpheaform, micronodular and basosquamous) BCC suggesting a role for MMP-14 in BCC invasion.[122] Stromal fibroblasts surrounding tumor islands of BCCs express MMP-14,[71] but the expression is not associated with MMP-2 expression.[78] MMP-21 expression is detected in cancer cells of aggressive sclerosing BCC subtype.[98]

The combination of MMPs in BCC has been studied in the BCC of eyelid. MMP-1, MMP-9, MMP-13 and TIMP-1 are expressed in the invading edge of epithelial tumor cells as well as in fibroblasts, inflammatory cells and endothelial cells. Expression of MMP-13 and TIMP-1 in tumor and stromal cells were in correlation, and TIMP-1 expression was associated with BCCs of morphea/sclerosing type indicating its role in BCC invasion, recurrence and poor prognosis.[123]

Expression TIMP-1 and TIMP-2 is detected in the stromal compartment of BCC, and the staining intensity of TIMP-1 was stronger in BCC compared to cSCCIS. Moreover, TIMP-2, but not TIMP-1, was expressed more abundantly in BCC compared to normal uninvolved skin.[84] Expression of TIMP-3 mRNA is detected in tumor cells of infiltrative basal cell carcinomas and in surrounding stromal cells in cSCCs.[100]

5 | INTERPLAY OF TUMOR CELLS AND FIBROBLASTS IN cSCC

5.1 | Pro-invasive effect of TGF-β

TGF-β can affect several cell types in the tumor microenvironment and promote cancer growth, invasion and metastasis.[124] TGF-β can act on the tumor cells directly and promote their capacity to remodel the surrounding ECM (Figure 1, Figure 2). In cSCC, both tumor cells and fibroblasts produce TGF-β.[125,126] Fibroblast-derived TGF-β can enhance proteinase expression and invasion of cSCC tumor cells.[125] Tumor cell- and fibroblast-derived TGF-β stimulates the expression of MMPs, for example MMP-13 and can this way increase release and activation of TGF-β from ECM.[126] This generates a positive regulatory loop leading to further TGF-β activation, tumor progression and invasion (Figure 2). Invasion of cSCC cells is potently stimulated by TGF-β via Smad and p38 MAPK signalling.[125,127,128] In addition, expression of MMP-13 promotes survival of both cSCC cells and stromal fibroblasts.[129–131]

5.2 | Anti-invasive effect of KGF

Keratinocyte growth factor (KGF, fibroblast growth factor-7 (FGF-7)) is produced by cells of mesenchymal origin and by epidermal γδ T cells.[132,133] KGF receptor (KGFR) is a splicing variant IIIb of FGF-receptor-2 (FGFR2-IIIb), and it is expressed by epithelial cells, including epidermal keratinocytes.[134,135] KGF is mitogenic for keratinocytes, and in normal wound healing, production of KGF by dermal fibroblasts is induced after injury in response to inflammatory mediators and growth factors, for example TNF-α, interleukin-1 (IL-1)and TGF-α.[136–138] KGFR is expressed also by cSCC cells, but they are unresponsive to the mitogenic effect of KGF.[139] In contrast, KGF reduces the invasion capacity of KGFR-positive cSCC cells and downregulates the expression of several genes linked to tumor progression and invasion, including MMP-13 and MMP-7 *via* ERK1/2 signalling pathway (Figure 4).[139] It is therefore conceivable that KGF serves as a suppressor of malignant phenotype of cSCC cells at the early stage of cSCC development. However, this tumor suppressive effect of KGF is compromised by downregulation and eventual loss of KGFR in the most aggressive SCC cells.

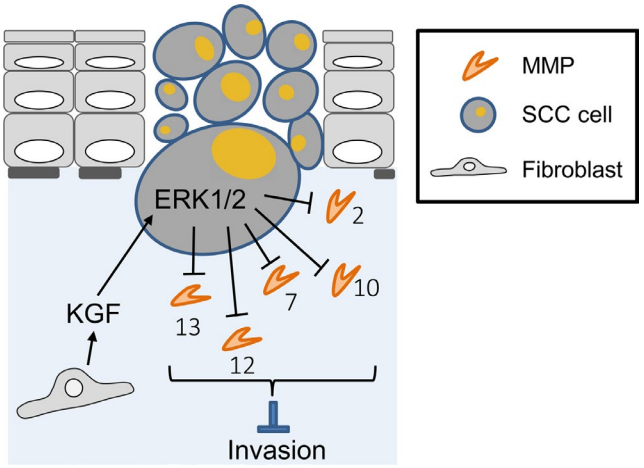

FIGURE 4 Keratinocyte growth factor (KGF) suppresses the malignant phenotype of cSCC cells. KGF is produced by stromal fibroblasts, and it decreases the invasion of cSCC cells by suppressing the MMP production. Numbers indicate the corresponding MMPs (MMP-13, -12, -7, -10 and -2)

6 | CONCLUSIONS AND FUTURE PERSPECTIVES

Although a small portion of primary cSCCs metastasize, the survival of patients with metastatic cSCC is poor, emphasizing the need for new therapeutic strategies, as well as biomarkers to predict tumor aggressiveness. Progression of cSCC involves interaction of several cell types in tumor microenvironment. Tumor-associated inflammatory cells and cancer-associated activated fibroblasts modulate microenvironment by secreting MMPs, which promote inflammation and remodelling of the surrounding stroma, providing a favourable environment for tumor growth. In this respect, targeting the interaction of tumor and the surrounding stroma may provide an interesting approach for cancer therapy. Accordingly, tumor microenvironment can modulate the efficacy of cancer therapies and resistance of tumor cells to therapies.[140] It is possible that targeting therapies to molecules modulating the tumor microenvironment could improve the efficacy of current therapies.[141] In this respect, cancer immunotherapy is a good example of therapies targeted to tumor microenvironment.[142]

The role of MMPs in cancer invasion and metastasis has encouraged development of small molecule inhibitors of MMPs for cancer therapy.[143–146] Several MMP inhibitors were initially shown to be effective in inhibiting growth, invasion and metastasis of malignant tumors in preclinical models, but none of these inhibitors were effective in clinical trials.[147,148] One explanation is that the molecules studied were broad-spectrum inhibitors of several MMPs and interfered with the normal physiological functions of specific MMPs, this way leading to various undesirable side effects.[149] In addition, distinct MMPs exert different functions in the microenvironment of malignant tumors, and a specific MMP can exert either tumor promoting or tumor suppressive functions.[150] It has become evident that due to high structural homology of the active domains of distinct MMPs, it is challenging to design selective MMP inhibitors targeted to the catalytic site. Therefore, new approaches for developing selective MMP inhibitors have been taken, including inhibitory monoclonal antibodies, and endogenous-like inhibitors such as the MMP prodomain and engineered TIMPs.[151] There is reason to expect that these next-generation MMP inhibitors may be developed to novel MMP-targeted cancer therapies also in KCs.

In conclusion, the microenvironment of cSCC is altered during tumor progression from AK to cSCCIS and finally to invasive and metastatic cSCC. Similarly, the microenvironment can promote the invasive capacity of BCC. Here, tumor cells, stromal fibroblasts and inflammatory cells remodel stromal ECM by secreting proteinases, which also regulate the activities of cytokines, chemokines and growth factors, and this way generate a tumor promoting environment. It is conceivable that the development of novel therapeutic approaches for metastatic cSCC and invasive BCC requires improved understanding of molecular mechanisms of tumor invasion and metastasis in these keratinocyte carcinomas.

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

PR, LN and VMK drafted the manuscript and designed the figures. All the authors have read the manuscript and have approved this submission.

ORCID

Pilvi Riihilä https://orcid.org/0000-0002-2934-0645
Liisa Nissinen https://orcid.org/0000-0002-6743-6736
Veli-Matti Kähäri https://orcid.org/0000-0003-2421-9368

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