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Inflammation in epithelial skin tumours: Old stories and new ideas

*Margareta M. Mueller**

Group of Tumor and Microenvironment, German Cancer Research Centre (DKFZ), Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany

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ABSTRACT

The essential contribution of inflammation to tumour development and progression has gained increasing acceptance. For epithelial skin cancer, the observation that tumours arise in sites of chronic irritation and inflammation dates back to 1828 and has stimulated a whole field of research. Chemically-induced mouse skin tumours requiring inflammatory agents such as 12-O-tetradecanoylphorbol 13-acetate (TPA) for tumour-promotion have greatly contributed to our understanding of multi-stage carcinogenesis and have given important insights into the functional interaction between inflammatory micro-environment and epithelial tumour, especially when used in combination with transgenic animals. Data from these and additional new model systems clearly emphasise that the tumour-promoting micro-environment is indispensable for tumour formation and progression. It strongly resembles the wound and is largely orchestrated by inflammatory cells allowing tumour cells to co-opt signalling molecules of the innate immune system to promote their growth, invasion and metastasis. Consequently, anti-inflammatory drugs are of great clinical interest in prevention and treatment of epithelial skin cancers.

1. Introduction

Inflammation is an age-old process that has proved essential for survival. As a crucial function of the innate immune system, it protects against pathogens by destroying infectious agents, gathers intelligence for the immune system by initiating specific and long-term immunity, and repairs damaged tissue. Acute inflammation is a rapid self-limiting process; however, it does not always resolve quickly, but may be maintained for a prolonged time and/or become chronic. There is increasing evidence that chronic, often subclinical, inflammation lies at the basis of many of the diseases of advanced age, such as heart attacks, Alzheimer's disease and cancer. The connection between inflammation and cancer has recently become 'hot news' by making the front page of *Time* magazine.[1] However, the association between inflammation and cancer was discovered much earlier. It was Virchow in 1863, who described for the first time the presence of a leukocytic infiltrate in tumour tissues and concluded that there ought to be a functional connection between inflammation and cancer.[2] Over the past decade our increasing understanding of the inflammatory environment of malignant tissues has supported Virchow's hypothesis,[3–5] yet the molecular and cellular mechanisms mediating the relationship between cancer and inflammation are still only partially resolved. This review will focus on the role of inflammation in development and progression of epithelial skin cancer, summarise the clinical and experimental evidence, and discuss potential consequences for therapy and prevention.

* Tel.: +49 6221 424 533; fax: +49 6221 424 551.
E-mail address: ma.mueller@dkfz-heidelberg.de.
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2. Physiological and pathological inflammation in the skin

To understand the contribution of the inflammatory microenvironment to the development and progression of epithelial skin cancer it is important to first understand how it contributes to physiological processes such as epidermal wound healing and infection (Fig. 1). In response to injury the tissue repair process is initiated immediately by the release of various growth factors, and low molecular weight compounds from the serum of injured blood vessels and from activated degranulated platelets. Platelets also participate in the formation of the blood clot that is composed of cross-linked fibrin, and of extracellular matrix proteins such as fibronectin, vitronectin and thrombospondin.[6–8] The fibrin clot serves as a barrier against invading micro-organisms, as matrix for invading cells and as reservoir of growth factors. In the course of platelet-induced haemostasis, platelets release platelet-derived growth factor (PDGF), transforming growth factor (TGF)-beta, platelet-activating factor and platelet factor 4 (PF-4). Together with bacterial products (if present) these growth factors are instrumental in stimulating chemotaxis of neutrophil granulocytes to the site of injury. Neutrophils arrive within a few minutes after injury, and contribute to the defence against micro-organisms by phagocytosis and production of proteinases and reactive oxygen species. In addition, they are an important source of cytokines/chemokines that are necessary for cell recruitment, activation and differentiation.[4,9] At the same time, some of these factors, such as interleukin (IL)-1-alpha, initiate the proliferation phase of wound repair by inducing keratinocyte proliferation factors, such as keratinocyte growth factor (KGF) and granulocyte–monocyte colony stimulating factor (GM-CSF), in stromal fibroblasts.[10] Following the transient neutrophil recruitment, and reaching a maximal number when neutrophils are declining, monocytes/macrophages are recruited to the sites of injury. They are again attracted by chemotactic factors including PF-4, PDGF, TGF-β, chemokines, macrophage inflammatory protein 1 (MIP-1) and the cytokines interleukin (IL)-1-beta and tumour necrosis factor (TNF)-alpha. After activation macrophages represent the main source of growth factors and cytokines such as TGF-α, TGF-β-1, PDGF, basic fibroblast growth factor (bFGF), IGF-I and -II, TNF-α and IL-1 that modulate tissue repair. In addition, they contribute to the remodelling of the extracellular matrix (ECM), and to angiogenesis by secreting proteases such as matrix metalloproteases (MMPs) and urokinase-type plasminogen inhibitor (uPA) and ECM-components such as thrombospondin-1.[4,11] As the third cell type of the innate immune system, mast cells contribute to the inflammatory response after tissue injury by releasing MMPs and serine proteases, heparin and heparanase, as well as various growth factors such as bFGF and vascular endothelial growth factor (VEGF)[12] and thus contribute to the early initiation phase of inflammation as well as to the late phase when leukocytes accumulate and wound healing takes place. This latter phase of wound healing is then characterised by migration of fibroblasts into the provisional wound matrix, where they are stimulated by IL-1-α and -β and TGF-β-1, -β-2, -β-3 to synthesise ECM components[4] and differentiate into myofibroblasts that facil-

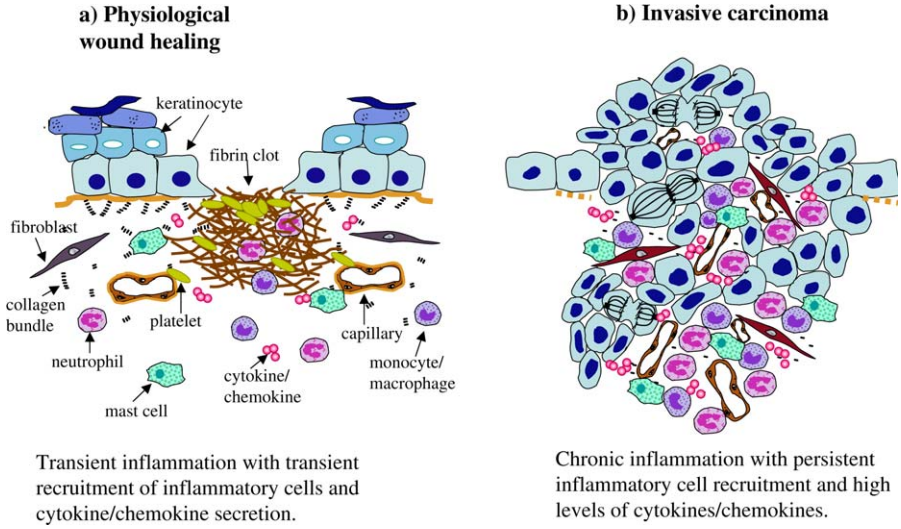

Fig. 1 – Physiological and pathological inflammation: (a) During wound healing or tissue injury the repair process is immediately initiated by the formation of a fibrin clot containing extracellular matrix (ECM) proteins and platelets. Degranulation of platelets provides the first 'wave' of growth factors, cytokines and chemokines that recruit inflammatory cells such as mast cells and neutrophils. Monocytes/macrophages reach maximal numbers when the neutrophil number is declining again. Together, these cells then orchestrate the inflammatory tissue response that is essential for the induction of angiogenesis and the healing/closure of the wound. Upon re-epithelialisation the inflammatory tissue response is down-regulated, resulting in a decreased level of inflammatory cytokines and reduction in the number of innate immune cells in the dermis. (b) In the tumour tissue the inflammatory reaction is 'chronic', with persistent recruitment of different inflammatory cell types into the tumour stroma as well as a constitutively high level of inflammatory cytokines and chemokines that is provided by tumour and stromal cells. By secreting proteases as well as angiogenic factors the inflammatory infiltrate critically contributes to angiogenesis, tumour invasion and metastasis.

itate wound contraction.[13] The final re-epithelialisation of the wound starts with the proliferation and migration of keratinocytes at the wound edge and requires the dissolution of the fibrin clot and a degradation of collagen by keratinocyte-derived uPA and MMPs.[6] Thus, besides the essential contribution of cell–cell and cell–matrix interactions to wound healing, the inflammatory response that is associated with the repair process has a key position in orchestrating a successful tissue repair. An extensive network of cytokines and chemokines controls this inflammatory response. However, while normal inflammation, e.g. during wound healing, is a rapid self-limiting process, deregulation of any of the factors can lead to abnormalities and, ultimately, pathogenesis. The profile and level of cytokines/chemokines that persists at sites of inflammation is important in the development of such pathologies (Fig. 1).

One example is the multi-potent growth factor TGF-β-1 that plays a central role during wound healing and inflammation. On the one hand it acts as a pro-inflammatory factor mediating leukocyte recruitment and regulation of MMP secretion and activation, yet it is also capable of suppressing these effects to mediate tissue repair.[14] As soon as this balance is tipped by a deregulated TGF-β expression, e.g. in basal cells of the skin of TGF-β-1 transgenic mice, the pro-inflammatory action of TGF-β-1 predominates and these mice develop a severe inflammatory skin disorder that resembles psoriatic lesions and is associated with the expression of additional inflammatory cytokines and chemokines such as interferon (INF)-gamma, IL-2, TNF-α, MIP-1-α and -β, MIP-2, and macrophage chemotactic protein-1 (MCP-1).[15] The relevance of TGF-β-1 in inflammatory skin diseases is further substantiated by its over-expression in disorders such as psoriasis.[16,17] A similar inflammation promoting role of TGF-β-1 and also -2 was observed in adult wound healing that is associated with scarring. By contrast, scar-free embryonic wound healing is dominated by the activity of TGF-β-3.[18–20] Interestingly, the main difference between adult and embryonic wound healing is the lack of inflammation in the embryo. Thus, scarring could be the price to pay for protection against infection that is conveyed by the inflammatory response[21] and this might be the reason for the successful use of anti-inflammatory steroids in the treatment of hypertrophic scars.[22] Studies in genetically modified mice that have deficiencies in their inflammatory response support this hypothesis. When these mice are wounded they do not scar and even heal faster[23–26] suggesting that in healing wounds less inflammation might be better. Similarly, Smad 3 knockout mice that are deficient in TGF-β signal transduction show an accelerated wound healing and decreased inflammatory response[24] as well as a reduced susceptibility to the development of epithelial skin cancer.[27]

3. Clinical evidence linking inflammation and epithelial skin cancer

Looking now at the clinical and epidemiological evidence for a connection between inflammation and epithelial skin cancer, we immediately encounter a highly controversial discussion concerning a potentially enhanced susceptibility for epithelial skin tumours in inflammatory skin disorders such as psoriasis. For psoriasis this controversy is based on numerous studies that show either an association of the disease with enhanced susceptibility for epithelial skin cancer (e.g.[28]) or a lack thereof (e.g.[29]). More recent studies clearly point out that it might be difficult to resolve this controversy at present, since many of the therapies that have been used in the treatment of psoriasis, such as ultraviolet (UV)-irradiation, or psoralen, have in the meantime been recognised as risk factors for the development of squamous cell carcinomas (SCC) and basal cell carcinomas (BCC).[30–33] A recent review by Nickoloff and colleagues discusses this controversy and a premature senescent phenotype of keratinocytes in psoriatic lesions that acts 'tumour-suppressive' as explanation for a potential lack of correlation between psoriasis and an increased risk for epithelial skin cancers.[34] However, despite the controversial discussion concerning psoriasis there is abundant clinical evidence for an association of chronic inflammation with epithelial skin tumours (Table 1). One of the earliest descriptions of this phenomenon is that of Marjolin in 1828, describing a relatively uncommon ulcerative condition associated with a thermal injury (Marjolin's ulcer) in which malignant transformation occurs within a chronic inflammatory focus.[35,36] A similar association of SCC development with inflammatory disorders in non-healing wounds has been described for lupus erythematosus, leg ulcerations, osteomyelitis,[37,38] perineal inflammatory disease,[38,39] ulcerative lichen planus[40] and epidermolysis bullosa of different aetiology.[41–43] More so, even areas of healed wounds with an established scar tissue are more susceptible to development of SCC and BCC.[44,45] However, inflammation is not only associated with the de novo development of epithelial skin tumours, but also seems to play an important role in their progression. A study by Berhane and colleagues suggests that progression of actinic keratoses to SCCs is preceded by a short inflammatory phase in the actinic keratosis. This is paralleled by an increase in the number of cells expressing detectable levels of p53 and Bcl-2 and a decrease in the number of cells expressing FasL, suggesting increasing resistance to cell cycle arrest and

Table 1 – Chronic inflammation or irritation associated with neoplasms

Pathological condition	Tumour type	Reference
Lupus erythematosus	SCC	[37]
Acne conglobata	SCC	[37]
Dissecting perifolliculitis of the scalp	SCC	[37]
Hidradenitis suppurativa	SCC	[37]
Acrodermatis chronica athrophicans	SCC, BCC	[37]
Osteomyelitis	SCC	[37,38]
Epidermolysis bullosa	SCC	[41–43]
Ulcerative lichen planus	Verrucous carcinoma (SCC)	[40]
Perineal inflammatory disease	SCC	[38,39]
Leg ulceration	SCC	[37]
Severe thermal injury	Marjolin's ulcer (SCC)	[35,36]
Burn scars	SCC, BCC	[44,45]

SCC, squamous cell carcinoma; BCC, basal cell carcinoma.

apoptosis.[46] Taken together, the association of inflammation with enhanced tumour formation and tumour progression is supported by a large number of clinical studies; however, these studies do not allow any insight in the cellular and molecular mechanisms that lie at the basis of the tumour and progression-promoting effect of inflammation in epithelial skin cancers.

4. Inflammation and skin cancer: experimental models

4.1. *Inflammation promotes skin carcinogenesis*

In order to elucidate these mechanisms, a number of animal models have been developed that have proven invaluable in understanding the contribution of the inflammatory process to tumour growth and progression. One of the oldest and maybe best-analysed animal models linking inflammation with the development of skin cancer is the classical tumour initiation and promotion scheme for the induction of mouse skin tumours. In this model, initiation is achieved by application of a very low dose of a genotoxic carcinogen that is by itself not sufficient to induce tumours in the experimental animal. However, it results in an initiated state of the epidermal keratinocytes, which frequently harbour one single genetic mutation (e.g. ras activation) and are more susceptible to subsequent genetic alterations. Tumour development only occurs when animals are subsequently treated with repeated doses of a tumour-promoting agent. Efficacy of tumour promotion is not linked to genotoxicity, since even the most potent tumour promoters do not cause gene mutations in the customary assay systems and initiate tumourigenesis only very weakly.[47] The most potent and most frequently used tumour promoters, the phorbol esters with 12-*O*-tetradecanoylphrobol 13-acetate (TPA) as a prototype, activate a series of protein kinase C (PKC) iso-enzymes and induce a pleiotrophic tissue response encompassing a strong inflammatory reaction.[48] Indeed, TPA treatment induces a reaction similar to the inflammatory wound reaction. It stimulates the enhanced expression of a wide variety of inflammatory wound factors, e.g. mediated through the activation of PKC-α, the up-regulation of TNF-α, MIP-1-α and MIP-2.[49] Accordingly, TPA treatment in tumour initiation can be replaced by repeated wounding as well as by injection of wound growth factors, such as TGF-β in combination with TGF-α.[50] By contrast, when using a phorbol ester that does not induce cutaneous inflammation, such as 4-*O*-methyl-12-*O*-tetradecanoylphorbol 13-acetate, a clear lack of tumour promotion could be demonstrated.[51] There are few tumour promoters that act without inducing an inflammatory response[52] and one has to keep in mind that the inflammatory reaction that is initially observed during TPA treatment is no longer maintained at the time of tumour development approximately 7 weeks after the start of treatment. Nevertheless, the concept of a tumour-promoting role for inflammation is supported by a large body of evidence. Accordingly, transgenic over-expression of 'pro-inflammatory' cyclo-oxygenase-2 (COX-2), a key enzyme in the synthesis of prostaglandins, sensitises mouse skin for carcinogenesis and abrogates the need for long-term treatment of TPA in addition to the initial 7,12-dimethylbenzanthracene (DMBA).[53] Transgenic over-expression of the inflammatory cytokine IL-1-α in basal keratinocytes also resulted in enhanced carcinoma formation. However, this effect was not observed after the DMBA/TPA two-stage carcinogenesis protocol, but only became apparent after repeated application of the genotoxic DBMA alone.[54] Both studies clearly suggest that inflammation induced by transgenes can replace the inflammation inducing phorbol ester in promoting tumour formation and demonstrate an essential role for the factors and signalling cascades that are associated with inflammation in tumour promotion. The tumour-and even progression-promoting effect of inflammatory cytokines is further substantiated by the enhanced tumour growth of IL-6-transfected human BCCs[55] as well as by the malignant progression that is associated with the expression of G-CSF and GM-CSF in HaCaT keratinocyte tumour cells.[56,57] Induction of G-CSF expression alone in previously benign factor-negative cells resulted in the progression from a cystic tumour showing no invasion into the surrounding host stroma to an invasive SCC. Co-expression of G-CSF and GM-CSF together further promoted progression to a highly malignant very fast growing and ultimately metastasising SCC type. This tumour progression was accompanied by a clearly enhanced and persistent inflammatory response of the host stroma.[58]

4.2. *Inhibiting inflammation inhibits epithelial tumour formation*

Conversely, inhibition of the tumour-promoting effect of TPA by chemical[59,60] or natural compounds[61,62] seems always to be associated with the inhibition of TPA-induced inflammation. As a consequence, the effect of anti-inflammatory drugs in chemoprevention of epithelial skin tumours has been studied extensively. Non-steroidal anti-inflammatory drugs (NSAIDs), e.g. inhibitors of COX-2, have been highly successful in preventing chemical carcinogen-induced skin tumours in the mouse. Together with the data for NSAID-mediated cancer prevention in colon cancer, this has been the encouraging basis for development of new non-steroidal anti-inflammatory components and the extensive testing of NSAIDs in cancer chemoprevention in humans.[47,63]

A more detailed analysis of the pathways and molecules involved in the tumour-promoting effect of inflammation has become possible through the use of knockout mouse models. Using this approach, the role of prostaglandins in tumour development was further substantiated by demonstrating a reduced susceptibility of mice deficient for the prostaglandin E2 receptor to DMBA/TPA-induced tumour formation.[64] Interestingly, TGF-β-1, which plays a crucial role in the inflammatory process during wound healing, up-regulates prostaglandin generation and COX-1 and -2 expression of mast cells.[65] As a consequence TGF-β-1-induced skin inflammation might significantly affect the role of TGF-β in skin carcinogenesis. In line with this, abrogation of TGF-β signalling by knocking out Smad3, confers resistance to chemical carcinogenesis.[27] Besides TGF-β-l, TNF-α is one of the most extensively and comprehensively studied pro-inflammatory cytokines in skin carcinogenesis. TNF-α plays an essential role during inflammation associated with wound

healing and was also shown to be essential for tumour promotion during chemical carcinogenesis. TNF-$\alpha^{-/-}$ mice are resistant to the development of benign and malignant skin tumours, whether induced by initiation with DMBA and promotion with TPA or by repeated application of DMBA. The resistance was associated with a clearly decreased inflammatory response in the dermis of the transgenic animals. Interestingly, the deletion of the TNF-α inducible chemokines CCL2 (MCP-1) also had a significant, albeit somewhat smaller, effect. Later stages of carcinogenesis were not affected by TNF-α as tumours in wild-type and TNF-$\alpha^{-/-}$ mice had similar rates of malignant progression.[66,67] Thus, the pro-inflammatory effect of TNF-α seems to be important for early stages of tumour promotion. Indeed, as shown by blockade of TNF-α with a neutralising antibody, it is the induction of TNF-α in skin keratinocytes during early stages of tumour promotion (0–6 weeks) that is critical for skin tumour promotion.[67] Further analysis of the TNF-α signalling pathways revealed an involvement of both TNF-α receptor subtypes, as well as of PKC-α and transcription factors of the AP-1 family in tumour promotion.[68,69] TNF-α signal transduction ultimately resulted in the activation of Ap-1 responsive genes, such as GM-CSF or MMP-9,[69,70] which promote inflammation and angiogenesis as well as proliferation and invasion of tumour keratinocytes. Taken together these studies allow us for the first time to follow the functional role of a pro-inflammatory, tumour-promoting cytokine from its initial activation to its ultimate effects on target gene expression.

5. Contribution of different inflammatory cells to tumour initiation and progression

Despite the tremendous progress that has been made in recent years in understanding the contribution of specific cytokines/chemokines and signalling pathways to the tumour-promoting effect of inflammation, it remains critically important not only to understand the molecules but also the cellular interactions that play a role in this process. Early studies in skin during chemically-induced carcinogenesis already pointed to an important role of an interaction between inflammatory cells and epithelial cells in this process. Farnoush and Mackenzie observed a clearly enhanced recruitment of mast cells below regions of epithelial hyperproliferation in DMBA/TPA-treated mouse skin, suggesting a contribution of the mast cells to the proliferative burst in epithelial cells.[71] The essential role of mast cells was further substantiated by studies in a transgenic model for de novo skin carcinogenesis, the K14-HPV16 mouse model. These mice spontaneously develop epidermal hyperplasia, followed by dysplasia and the formation of papillomas. Progression to malignant SCCs is observed in approximately 20% of the mice.[72] In this system, mast cells infiltrate hyperplastic and dysplastic lesions as well as the invasive front of tumours. There they contribute to the activation of a tumour supporting stroma and to angiogenesis by releasing mast cell chymase and tryptase as well as by activating pro-MMP-9, which is provided by mast cells themselves as well as by neutrophil granulocytes (Fig. 2(a)).

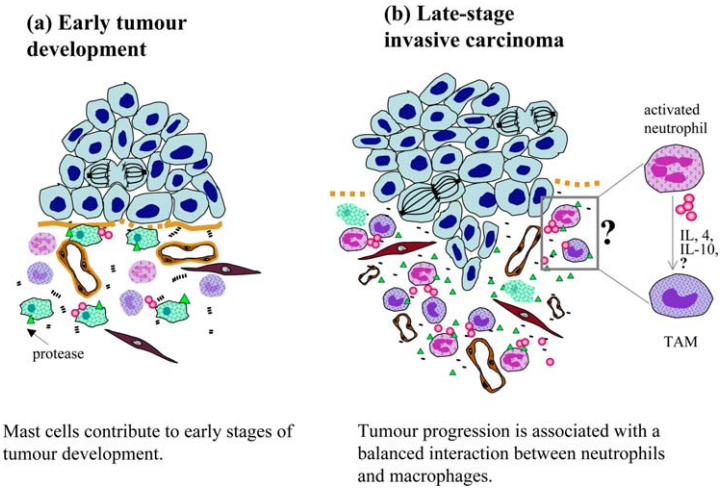

Fig. 2 – Differential roles of specific inflammatory cells during tumour development and progression. (a) During early stages of skin tumour development, mast cells, macrophages and neutrophils are recruited to the activated stroma. Mast cells are found abundantly in regions of chemically-induced epithelial hyperproliferation and papillomas. Their essential contribution to tumour development comprises the secretion of mast cell proteases and matrix metalloproteases (MMPs), as well as polypeptide growth factors, such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF) and others. The proteases and growth factors support the persistent activation of the tumour stroma and promote angiogenesis as well as tumour invasion, thereby creating a permissive environment for further tumour progression. (b) Benign tumours show a transient recruitment of neutrophils and a persistent macrophage recruitment, resulting in an imbalance between both cell types in late stages of tumour transplants. Progression to malignant and invasive squamous cell carcinomas (SCCs) is associated with an enhanced and persistent recruitment of neutrophils in addition to enhanced macrophage recruitment. Both cell types reach similar numbers in the stroma of malignant tumour, providing the possibility of a balanced interaction via soluble factors and cytokines. We hypothesise that this balanced interaction might contribute to the differentiation of macrophages to tumour-associated macrophages (TAMs), and thus promote malignant progression.

The essential role of both mast cells as well as stromal MMP-9 for tumour development is demonstrated by crossing K14-HPV16 mice with mice deficient for either mast cells (KitW/KITWWv) or MMP-9.[73,74] Tumour incidence was clearly decreased in both models. In addition, lack of MMP-9 was associated with delayed activation of angiogenesis in the stroma of the lesions. Interestingly, the deficiency of MMP-9 could not be rescued by epithelial keratinocytes, which are in principle capable of expressing MMP-9 (reviewed in[75,76]), but required the presence of MMP-9 proficient haematopoietic cells, as was demonstrated by transplantation of bone marrow from MMP-9 wild-type mice.[74] Thus, mast cell contribution clearly seems critical for the initial development of malignant skin SCCs (Fig. 2(a)) and there is recent evidence that B lymphocytes play an important role in recruiting mast cells to the tumour stroma.[77] However, the inflammatory reaction that is characteristic for the activated stroma of skin SCCs also encompasses additional cell types such as neutrophils and macrophages. Both were shown to be recruited upon tumour induction by chemical carcinogenesis[78] and there is evidence that it is the TPA-mediated induction of PKC-α and the resulting expression of inflammatory and chemotactic factors, such as GM-CSF, that is necessary for their recruitment.[79,80] The functional contribution of GM-CSF as

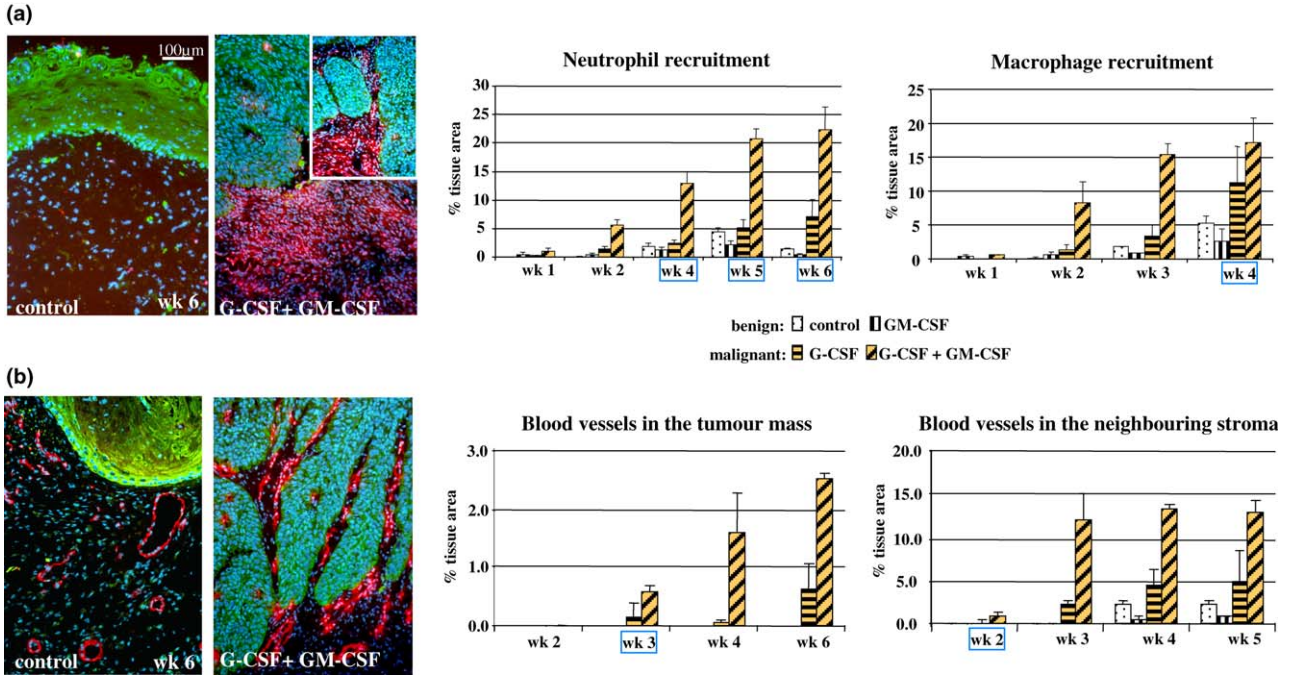

Fig. 3 – Persistent neutrophil recruitment and persistent angiogenesis are associated with malignant progression. (a) Kinetics of inflammatory cell recruitment: benign and malignant HaCaT keratinocyte tumours were transplanted in the matrix-inserted surface transplantation chamber to analyse the kinetics of stromal activation and angiogenesis. Tumour transplants were analysed 1, 2, 3, 4, 5 and 6 weeks after transplantation. Cryo-sections of tumours from benign (control) and malignant (G-CSF + GM-CSF) transplants were stained against keratin (green) to visualise tumour cells, granulocytes (red), and nuclei (blue) in order to determine neutrophil recruitment into the tumour vicinity. While benign transplants show almost no neutrophils at late stages of transplantation (6 weeks), neutrophils are abundant in the stroma of malignant transplants. Quantification of neutrophil recruitment, and similarly of macrophage recruitment, in sections stained for macrophages is shown as percent tissue area. While granulocyte recruitment in benign tumours (*bars with white background*) is transient with a significant decrease in neutrophil number in late tumour transplants between week 5 and 6 neutrophil recruitment is persistent in malignant tumour transplants (*bars with orange background*), resulting in a continuously increasing number of neutrophils in the tumour vicinity. Macrophage recruitment is continuous in benign as well as malignant transplants. However, macrophage number is clearly enhanced in the stroma of malignant transplants. Due to the transient recruitment of neutrophils in benign transplants there are significantly less neutrophils than macrophages in late stages of benign tumours (week 4 and later). By contrast, the number of neutrophils and macrophages are approximately even in malignant transplants, allowing a balanced interaction between both cell types. (b) Kinetics of angiogenesis: cryosections of tumours from benign (control) and malignant (G-CSF + GM-CSF) tumour transplants were stained against keratin (*green*) for tumour cells, CD31 for vessels (*red*), and nuclei (*blue*) to determine the kinetics of angiogenesis. Quantification of blood vessels in the tumour-neighbouring stroma or in the tumour mass – only for malignant tumours with reciprocal invasion of vessels into the tumour and tumour cells into the underlying stroma – is shown as percent tissue area. The onset of angiogenesis follows the recruitment of inflammatory cells with a delay of approximately 1 week, obeying the same kinetics as neutrophil recruitment, i.e. transient in benign tumour transplants with vessel enlargement and maturation in late tumour stages and persistent in malignant tumour transplants.

well as of G-CSF to tumour progression and the recruitment of the inflammatory infiltrate in epithelial skin tumours was recently demonstrated in the HaCaT model for tumour progression of skin SCCs. In a matrix-inserted surface, transplantation model specifically developed for analysing the kinetics of tumour stroma interaction,[81,82] benign HaCaT tumour cells induce a persistent recruitment of macrophages into the vicinity of the tumour epithelium, while neutrophil recruitment remains transient (Fig. 3(a)). This inflammatory cell recruitment is highly similar to the initial inflammatory reaction during wound healing. Upon malignant progression e.g. induces by the co-expression of G-CSF and GM-CSF in the tumour cells macrophage recruitment is earlier and enhanced and macrophages now invade the tumour epithelium. At the same time neutrophil granulocyte recruitment is also accelerated and becomes persistent, resulting in a progressive accumulation of neutrophils in the tumour vicinity. This ultimately results in a balance between the number of macrophages and neutrophils in the tumour stroma. By contrast, there is a clear excess of macrophages in the stroma of benign tumours (Fig. 3(a)). Accordingly, an accelerated and enhanced recruitment of macrophages alone, followed by a later onset of a transient neutrophil recruitment that was induced by selective expression of GM-CSF in the benign tumour cells, is associated with initial tumour growth followed by tumour regression. Interestingly, the persistent neutrophil recruitment precedes the induction of a persistent angiogenesis that is a prerequisite for malignant tumour growth by approximately 1 week (Fig. 3(b)).[58] Neutrophils clearly promote angiogenesis and tumour invasion by secreting MMP-9.[83] In addition, they might contribute to a tumour-promoting inflammatory infiltrate by modulating the phenotype of the macrophages in the tumour vicinity via the secretion of cytokines or growth factors (Fig. 2). The differentiation of macrophages to tumour-associated macrophages (TAMs), a cell type that is oriented towards angiogenesis, and tissue remodelling (M2 phenotype) rather than being classically pro-inflammatory (M1 phenotype), is induced by IL-4 and IL-10 (reviewed in[84]). The functional contribution of these TAMs to promoting tumour progression includes the expression of a number of proteases, such as uPA and MMPs, which remodel the ECM, generating reactive cleavage products of ECM molecules and activating pro-angiogenic factors, such as VEGF and others. In addition, TAMs secrete pro-inflammatory cytokines and chemokines that further enhance stroma inflammation, as well as angiogenic factors, such as VEGF and bFGF, thereby supporting the establishment of an activated angiogenic and tumour-supporting stroma.[84,85] There is recent evidence that neutrophil granulocytes can modulate the phenotype of macrophages by suppressing their inflammatory phenotype through the secretion of soluble, but as yet unidentified products. [86] In addition, dermal neutrophils have been shown to express IL-4 and IL-10 in response to UV irradiation[87,88] and thus might be capable of regulating TAM differentiation via the secretion of the appropriate modulatory cytokines. Taken together, the studies presented here suggest that mast cells might have an important role in the initial stages of tumour development (Fig. 2), while the interaction of granulocytes and macrophages might be necessary for progression to a malignant tumour phenotype (Fig. 2).

6. Conclusion

While there are some studies that either show a lack of association between inflammation and epithelial skin cancer, e.g. for psoriasis,[34] or even a reduced tumour incidence of tumours in mice selected for maximal acute inflammatory responsiveness,[89] the predominant evidence clearly supports a tumour-promoting role of inflammation in the development and progression of epithelial skin cancer. Non-melanoma skin cancers (BCC and SCC) are the most frequent tumours worldwide, and their incidence is still increasing. In addition, immunosuppression in organ transplant patients strongly contributes to the increase in skin cancer incidence, being 65–250 times more frequent than in the general population. Often these patients suffer from a second and third lesion and the severity of these tumours is linked to their number.[90] Thus, new strategies for the prevention and treatment of these tumours should be an important focus in skin cancer research. In light of the essential role of inflammation in epithelial skin cancer, anti-inflammatory agents might open a new and promising avenue in cancer prevention. There are successful applications of TNF-α inhibitors and NSAIDs in mouse models of skin carcinogenesis,[63,67] and NSAIDs have shown efficacy in the treatment of skin tumour precursor lesions, such as actinic keratosis.[91] In addition, human trials using selective COX-2 inhibitors are currently in progress and should offer exciting information regarding the chemopreventive function of anti-inflammatory drugs.

Conflict of interest statement

None declared.

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