

Dermal mast cells affect the development of sunlight-induced skin tumours

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Abstract: Ultraviolet (UV) radiation contained in sunlight is considered a major risk in the induction of skin cancer. While mast cells are best known for their role in allergic responses, they have also been shown to play a crucial role in suppressing the anti-tumour immune response following UV exposure. Evidence is now emerging that UV may also trigger mast cell release of cutaneous tissue remodelling and pro-angiogenic factors. In this review, we will focus on the cellular and molecular mechanisms

by which UV recruits and then activates mast cells to initiate and promote skin cancer development.

Key words: immunosuppression – mast cells – skin cancer – ultraviolet radiation

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Introduction

Ultraviolet (UV) radiation in sunlight is carcinogenic and considered a major risk factor in the induction of skin cancer, especially following long-term chronic exposure (1–4). Despite increased awareness of the risks associated with exposure to UV, skin cancer incidence continues to rise faster than any other cancers, and melanoma is now one of the most common cancers seen in young people (those aged <50) (5). Exposure to UV not only results in DNA damage, but also in immune suppression, both of which are required for the development of skin cancer (6,7). This makes sunlight one of the most significant environmental carcinogens humans are exposed to. Currently, the mechanisms underlying UV-induced suppression of the anti-tumour immune response remain unclear.

In humans (8–10) and mice (11), UV has been shown to impair the host immune system, both locally at the site of exposure as well as systemically so that antigen applied to a skin site that has not directly received UV induces tolerance rather than immunity (11,12). In 1977, Fisher and Kripke experimentally documented the association between UV-induced immunosuppression and skin cancer development. In this study, UV-induced skin tumours failed to grow when transplanted into syngeneic non-irradiated mice, indicating that UV-induced tumours were immunogenic. In contrast, when the recipient mice were exposed to UV prior to receiving the transplant, the tumours were able to grow (13,14). Thus, UV suppression of anti-tumour immune responses enables the outgrowth of skin tumours. The significance of this is best illustrated by the abnormally large numbers of skin tumours that arise in immunologically suppressed transplant patients (15,16).

A number of cells participate in the skin immune response and are, therefore, targets of UV-induced immune suppression. In the epidermis, these include Langerhans cells (LC) and keratinocytes. Deeper within the dermis, fibroblasts, endothelial cells, dendritic cells (DC) and mast cells have all been shown to be involved in

UV-immunosuppression. Indeed, experiments showing that mast cell-deficient mice are resistant to the immunosuppressive effects of UV radiation strongly support the idea that mast cells in particular are crucial for UV-induced suppression of anti-tumour immune responses (17). A number of excellent reviews have been written within the past 10 years on the role of mast cells in promoting tumour growth (18,19), particularly skin cancer (20). This review will focus specifically on UV-induced skin tumour development in the context of recent discoveries on the cutaneous effects of UV on mast cells that arm them with potent immune modulating and tumour-promoting properties.

Mast cells

Immature bone marrow-derived mast cell precursors migrate from the blood into vascularized tissues and serosal cavities where they differentiate and mature into distinct mast cell phenotypes. The presence of stem cell factor (SCF), interleukin-3 (IL-3), IL-4 and IL-9 within these peripheral sites plays an important role in determining the ultimate phenotype of these tissue resident, mature mast cells. Mast cells are strategically located close to the outside environment and are found in abundance within the skin, airways and digestive tract (21). This widespread peripheral tissue distribution suggests mast cells have an important role to play in the defense against infiltrating pathogens as well as toxins (22) and other environmental insults such as solar UV (23). Mast cells release histamine and other inflammatory mediators in response to cross-linking of their IgE receptors and as such have traditionally been associated with allergic-type inflammation. However, there is now increasing evidence that mast cells perform important immunoregulatory functions via the release of cytokines and other mediators. For example, mast cells are critical in maintaining homeostasis, protecting the host from sepsis and in certain circumstances may even modulate regulatory T cell activation (24,25). Activation of mast cells via FcεR1α cross-linking (26) is the primary trigger for the rapid release of their granules by exocytosis. However, in some cases, mast cells selectively release their

Table 1. Mast cell-derived mediators¹

Mediator type	Examples
(i) Preformed mediators in Granules	Histamine, serotonin (in mice), proteoglycans (chondroitin sulphate, heparin sulphate), neutral proteases (chymase, serine proteases, tryptase, carboxypeptidase A)
(ii) Lipid-derived mediators	Platelet-activating factor, prostaglandin E₂ (PGE₂) Leukotriene B ₄ , Leukotriene C ₄ , HETES, and prostaglandin D ₂
(iii) Cytokines and growth factors	IL-4, IL-5, IL-6, IL-10, IL-12, IL-13, IFN-γ, TNF-α, NGF IL-2, IL-3, IL-8, IL-9, IL-11, IL-14, IL-15, IL-16, IL-17, IL-18, IL-22, IL-33, GM-CSF, IFN- α , IFN- β , LIF, CSF, MIF, SCF, TGF- β 1 ² , TSLP, EGF, VEGF ²
(iv) Chemokines	CCL1, CCL2, CCL3, CCL3-L1, CCL4, CCL5, CCL7, CCL8, CCL11, CCL13, CCL16, CCL17, CCL20, CCL22, CXCL1, CXCL2, CXCL3, CXCL10, CXCL11, CXCL12
(v) Other Mediators	Nitric oxide, superoxide, substance P, matrix metalloproteinase-9 , corticotrophin releasing factor

¹Mast cell products implicated in UVR-induced immunosuppression appear in bold. ²Mediators are also likely to have additional pro-tumorigenic properties (37,47,162,163).

mediators without degranulating, as originally reported for serotonin (27). Signalling through pattern recognition receptors (28) or products of complement activation binding to specific receptors on the surface of mast cells (29) may also trigger the release of a number of mediators. We have classified these into five groups (Table 1): (i) preformed mediators stored in granules such as histamine, serotonin, proteases; (ii) *de novo* synthesized lipid mediators such as prostaglandins and leukotrienes; (iii) cytokines and growth factors; (iv) chemokines; and (v) other mediators including reactive oxygen species, metalloproteinases and neuropeptides.

The IgE-independent, alternative pathway of mast cell mediator release may be particularly important as it has frequently been observed in mast cell infiltrating areas of chronic inflammation and tumours (30). Indeed, this appears to be an important mechanism by which mast cells release tumour-promoting cytokines and lipid mediators, particularly in the early stages of cancer initiation. For example, mast cell degranulation does not appear to be a major event in the development of benign adenomatous polyps (31). As yet, the precise cellular and molecular mechanisms involved in the recruitment and activation of mast cells by cancer causing UV radiation are relatively unknown.

Role of mast cells in UV-induced immune suppression and photocarcinogenesis

Mast cells function as immunomodulators

The ability of mast cells to release such a wide variety of inflammatory mediators enables them to modulate the recruitment, survival, development, phenotype and function of a large range of immune cells including granulocytes, monocytes/macrophages, DCs, T cells, B cells and natural killer (NK) cells (25). It has recently become clear that mast cells also participate in immunoregulation as shown by Lu et al. (24) who demonstrated that mast cells interact with CD4⁺CD25⁺FoxP3⁺ regulatory T cells (T_{Regs}) to promote skin allograft tolerance. However, it was research performed by Hart et al. (17) on UV-immunosuppression that established the first link between mast cells and immunoregulation.

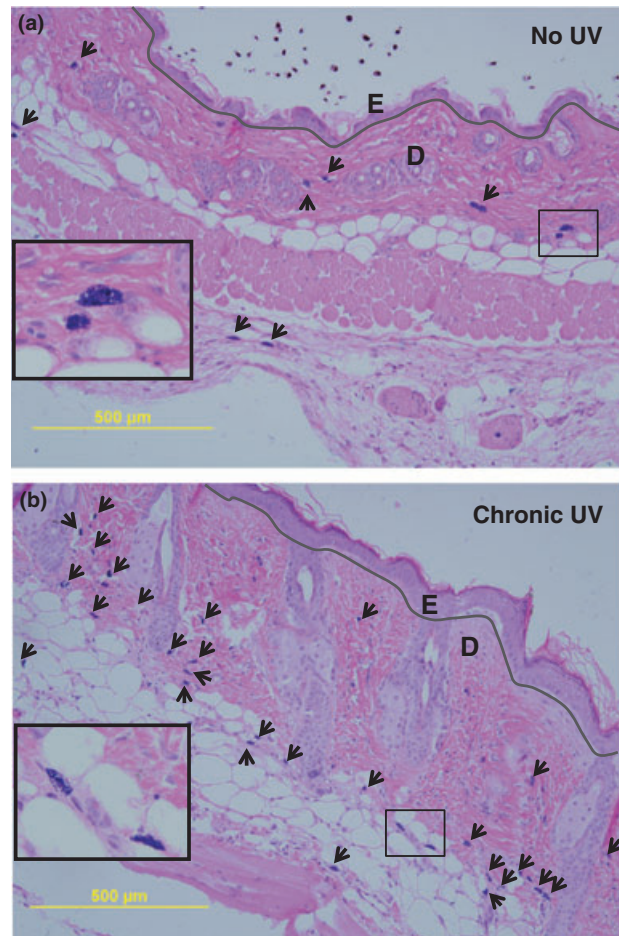


Figure 1. Histological section of dorsal skin from chronically UV-irradiated C57BL/6 mouse (250 mJ/cm² UVB four times per week for 6 weeks). Mast cells were identified by staining with Toluidine-blue. Skin architecture was revealed with H&E. (a) Control-unirradiated skin. (b) UV-irradiated skin. Mast cells in the dermis and subcutaneous fatty tissue have been highlighted with arrows (objective $\times 10$), epidermis (E), dermis (D).

In this elegant and ground-breaking study, UV was unable to suppress the immune response of mutant *W^f/W^f* mice that lack mast cells. Reconstitution of these mast cell-deficient mice with wild-type bone marrow-derived mast cells restored the ability of UV to induce immune suppression (17). Thus, mast cells are required for UV-induced immune suppression. The immunomodulatory function of mast cells is likely to be important for the development of skin tumours because mast cell density in human skin correlates with susceptibility to both melanoma (32) and non-melanoma (33) skin cancers.

UV recruits mast cells into the skin

Susceptibility to UV-immunosuppression predicts whether a patient is at risk of developing skin cancer (34), and higher numbers of dermal mast cells correlates with greater sensitivity to UV-immunosuppression (17,35). Compounding this effect is the fact that exposure to UV increases mast cell density in the dermis (36,37). Indeed, compared to protected skin sites, chronically sun-exposed human (38) and mouse skin contains significantly higher mast cell densities (Fig. 1), although whether this is in response to

sunlight itself or an intrinsic property of these skin sites (39) is unknown. This UV-induced mast cell recruitment is likely to prevent excessive UV-induced inflammation (40) and may explain the therapeutic effect of phototherapy in polymorphic light eruption (PLE) patients (41,42). However, although patients with skin cancer are significantly less likely to develop PLE (43), whether this is because of a lack of mast cell-mediated UV-induced immune suppression remains to be determined. While UV-induced mast cell recruitment will increase immune suppression and susceptibility to skin cancer, the precise mechanism by which UV recruits mast cells to sites of irradiation is not clear. UV-induced expression of SCF (c-kit ligand) (36) or CCL5 [regulated on activation normal T cell expressed and secreted (RANTES)] (44) may be involved as mast cells express the receptors for both SCF and CCL5, and both of these cytokines are mast cell chemoattractants (45). Considering the range of inflammatory mediators and cytokines that mast cells have been shown to produce (Table 1), the accumulation in UV-exposed skin of mast cells with immunomodulatory functions is likely to be important for both immune suppression and skin tumour development. This is supported by well-established observations that the presence of high numbers of mast cells correlates with poor prognostic outcomes for many tumour types including lymphoma, colon cancer, basal and squamous cell carcinomas as well as melanoma (46).

Factors released in the skin following UV radiation activate dermal mast cells

Following irradiation, the resident and newly recruited mast cells are exposed to a variety of UV-induced mediators produced by other cells in the skin that activate them to assume an immunoregulatory phenotype. In this way, dermal mast cells attune themselves to the environment by responding to the UV-induced conditions in which they find themselves. This has led some to refer to mast cells as 'tunable' immunoregulatory cells (47).

Cis-urocanic acid

Trans-urocanic acid (UCA) is abundantly expressed in skin and upon exposure to UV is rapidly isomerized to the immunosuppressive *cis*-UCA isoform (Fig. 2) (48). Similar to the effects of UV itself (17), *cis*-UCA is unable to suppress the cutaneous immune response of mast cell-deficient mice (49). The receptor for *cis*-UCA has been identified as the 2A serotonin receptor (5-HT_{2A}R) (50), which is expressed by mast cells (51). Using a human skin organ culture model, Wille et al. (52) showed that *cis*-UCA indirectly activates mast cells, although when mast cells were directly exposed to *cis*-UCA, they did not degranulate (53), but assumed an immunomodulatory phenotype (49). The mechanism of this indirect mast cell effect may be via the activation of cutaneous nerve fibres to release mast cell-activating neuropeptides (49).

Neuropeptides and nerve growth factor

UV exposure induces the release of a large variety of neuropeptides from cutaneous nerve fibres including calcitonin gene-related peptide (CGRP), substance P (SP), alpha-melanocyte-stimulating hormone (α -MSH) as well as nerve growth factor (NGF) (54). Mast cells express receptors for all of these mediators and are strategically located close to nerve fibres and blood vessels, enabling them to rapidly respond to the release of these factors. Niizeki et al. showed that CGRP itself is immune suppressive. CGRP antagonists blocked UV-induced immune suppression (55). This

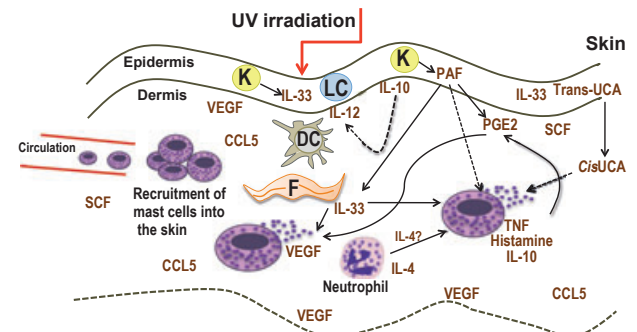


Figure 2. Role of mast cells in UV-induced immune suppression in the skin: DNA damage triggered by UVR may be responsible for altering Langerhans cell (LC) function via the production of soluble immunosuppressive mediators such as IL-10 from keratinocytes (K). An IL-10 induced decrease in IL-12 production by LC results in altered APC activity (164). UVR also triggers the isomerization of *trans*-urocanic acid (UCA) present in the outermost layer to *cis*-UCA. This is thought to mediate immune suppression through binding to serotonin receptors found on DC, T cells and B cells (50). *cis*-UCA formation is also thought to modulate mast cell (MC) activity (49) and has been linked to MC degranulation (52). Triggers for mast cell recruitment to sites of irradiation may include CCL5, SCF or VEGF. Keratinocyte production of PAF (165) is responsible for inducing prostaglandin E₂ (PGE₂). It is also believed that histamine, which may or may not be MC derived, also stimulates prostanoid production leading to downstream immune suppression (166). IL-4 is upregulated post-UV exposure (64) and is essential for normal mast cell development (63). Neutrophils are recruited into UV-exposed skin and may be responsible for additional IL-4 production (62). UVR also triggers the upregulation of IL-33 in epidermal keratinocytes (K) as well as dermal fibroblasts (F) (77).

group went on to show that the target of CGRP was a mast cell as these observations were lost in cKIT-mutant W/W^v-mast cell-deficient mice. With regards to NGF, it not only acts as a potent mast cell chemoattractant (56,57), but can also induce the release of IL-6 and prostaglandin E₂ (PGE₂) (58), both of which are well-known mediators of UV-induced immune suppression (59,60). In contrast to NGF, SP-treated mast cells degranulate and also produce significant amounts of CCL5, monocyte chemoattractant protein-1 (MCP-1) and IL-8 (61). This body of evidence suggests that UV-induced neuropeptides suppress anti-tumour immunity at least in part by acting on mast cells which then release immune suppressive cytokines and chemokines that attract inflammatory cells.

Interleukin-4

It is likely that multiple UV-induced signals are required for mast cell activation with IL-4 being another candidate. High expression of IL-4 has been observed in UV-exposed human skin, which was associated with the infiltration of neutrophils (62). Mice deficient in IL-4 are resistant to UV-induced suppression of cutaneous immune responses, and while their dermal mast cell density is normal, their mast cells have a defective response to UV (63). While IL-4 is abundantly produced upon UV exposure (64) and mast cells express IL-4 receptors (65), whether mast cells are the direct target of UV-induced IL-4 is not known. However, being a well-known and potent mast cell-activating cytokine (66), UV-induced IL-4 is likely to have a profound effect on the immunoregulatory phenotype assumed by dermal mast cells.

Vitamin D₃

Upregulation of the anti-inflammatory cytokine IL-10 by mast cells in response to UV is thought to be important in limiting the inflammatory effects of UV on the skin (40). However, IL-10 is also immune suppressive. Indeed, IL-10 deficient mice are resis-

tant to UV-induced immune suppression (67). This is likely to be important for UV-induced skin cancer development because these IL-10 knockout mice fail to develop any skin tumours (67). However, the exact mechanism that promotes mast cell IL-10 production in UV-exposed skin is still not completely known. Recent work by Biggs et al. (68) reported that vitamin D₃ receptors (VDRs) expressed by mast cells are required for their UV-induced IL-10 secretion. Wild-type mast cells cultured with biologically active vitamin D₃ (1 α ,25[OH]₂D₃) increase their expression of IL-10 mRNA and protein. However, mast cells derived from VDR-deficient mice failed to do so (68). The significance of these results were revealed when it was shown that mast cell-deficient mice engrafted with VDR-deficient bone marrow-derived mast cells were highly susceptible to the inflammatory effects of chronic UV exposure (68). In contrast, mast cell-deficient mice receiving wild-type (VDR⁺) mast cells were protected. Hence, interactions between VDR-expressing dermal mast cells and the active form of vitamin D₃ are involved in the activation of IL-10-producing immunomodulatory dermal mast cells following UV irradiation. This observation may explain in part the recently described therapeutic effects of vitamin D₃ analogues in PLE patients (69) who develop exaggerated inflammation upon exposure to UV.

Interleukin-33

IL-33 (IL-1F11, NF-HEV) is a member of the IL-1 family of cytokines (70) that binds to the IL-33 receptor (ST2) expressed on the surface of a number of immune cells including mast cells (70,71). IL-33 itself is expressed by a range of different cell types in both humans and mice (70), including epithelial cells, fibroblasts and endothelial cells (70,72). IL-33 modulates immune responses by binding to ST2, which triggers the production of Th2-related cytokines. Consequently, IL-33 has been shown to play an important role in modulating the immune response during atherosclerosis (73), infection (74) and asthma (75). IL-33 is upregulated in the skin during inflammatory events. For example, IL-33 is increased in the skin of psoriasis patients (76). We recently discovered that although resting, sun-protected skin does not express IL-33, it is significantly upregulated by keratinocytes and dermal fibroblasts upon exposure to an inflammatory dose of UV (77). UV-induced skin tumours that evade immunological destruction also produced significant amount of IL-33 (77). This upregulation of IL-33 was required for the suppression of Th1-immunity as neutralizing antibodies prevented UV-immunosuppression *in vivo*. This is likely to be important for skin tumour growth because a robust Th1 immune response is required for the prevention and control of tumour growth (78). Indeed, it is now well accepted that UV causes skin cancer in part through its ability to suppress Th1 immune responses (11,60). The exact mechanisms by which UV-induced IL-33 modulates Th1 immunity are unknown. The IL-33 receptor, ST2, is highly expressed by mast cells (70) and stimulation of bone marrow-derived mast cells with recombinant IL-33 induces production of immune suppressive cytokines like IL-10 (71) and IL-13 (Fig. 3). Our results are consistent with previous reports (79,80) and implicate mast cells as one of the main cellular targets for UV-induced IL-33-mediated immune modulation.

High amounts of mast cell-derived IL-13 together with IL-4 and IL-33 in UV-irradiated skin are likely to alter the activation state of macrophages that infiltrate UV-exposed skin (81). IL-13 is well known for its ability to polarize macrophages towards

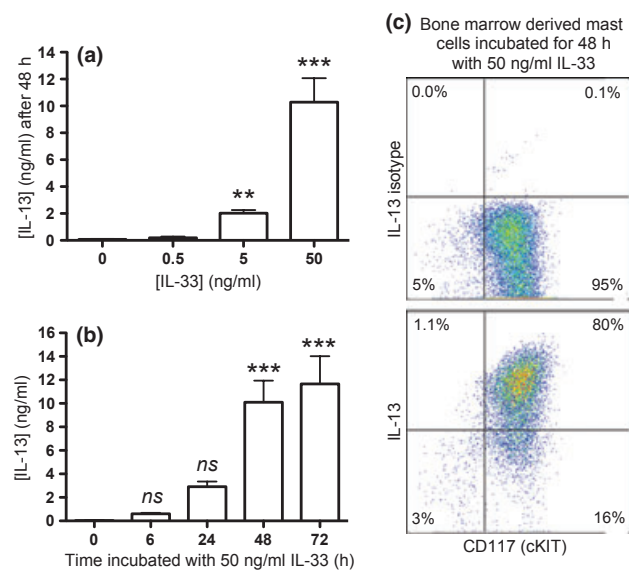


Figure 3. IL-33 induces the secretion of IL-13 by bone marrow-derived mast cells *in vitro*. Bone marrow stem cells from the femurs and tibiae of 6–7-week-old C57BL/6 mice were cultured in complete RPMI 1640 medium supplemented with IL-3 (10 ng/ml) and SCF (10 ng/ml) at 10^6 cells/ml. After 5 weeks of culture, >95% of cells were determined to be mast cells by flow cytometry (Fc ϵ R1 α ⁺CD117⁺). At this time, the mast cells were washed twice in complete RPMI (cRPMI) prior to culture at 0.5×10^6 cells/ml in cRPMI supplemented with recombinant murine IL-33 (a; 0–50 ng/ml for 48 h, or b; 50 ng/ml for 0–72 h). IL-13 levels in the cell culture supernatants were determined by ELISA (Peprotech, Rocky Hill, NJ, USA), with duplicate readings averaged for three individual samples in each experiment. Results are expressed as means $\pm$ S.E.M. for two separate experiments. *** P < 0.01, *** P < 0.001 compared to respective untreated (0 h or 0 ng/ml) control (one-way ANOVA with Dunnett's multiple comparison test). c; bone marrow mast cells were incubated with 50 ng/ml IL-33 for 48 h at which point intracellular flow cytometry was used to detect the presence of IL-13 in the IL-33-treated mast cells.

alternatively activated immune suppressive M2 macrophages (82), a process that is amplified by IL-33 (83). We hypothesize that in the presence of IL-4, IL-13 and IL-33, skin-infiltrating macrophages are polarized away from classically activated, tumour-destroying, IFN γ -secreting M1 macrophages towards the M2 phenotype. The ability of alternatively activated M2 macrophages to suppress anti-tumour immunity together with their pro-tumorigenic effects on tumour cells and the surrounding stroma (82) suggests that this is likely to have a profound effect on UV-induced skin tumour growth, immune evasion and metastasis. Our observations of high numbers of activated tumour-associated macrophages (TAMs) in progressor but not in regressor UV-induced skin tumours support this hypothesis (84), as does more recent evidence that human squamous cell carcinomas are also infiltrated by alternatively activated M2 macrophages (85).

Consequences of UV-induced mast cell recruitment and activation on anti-tumour immunity

Histamine

Following the UV-induced recruitment and activation of mast cells, a cascade of cellular and molecular events conspires to create an immune privileged microenvironment conducive of skin tumour growth. For example, mast cell-derived histamine appears to be an important rate-limiting step in UV-induced immunosuppression (86,87). Histamine can suppress cutaneous immune responses when injected into mast cell-deficient mutant *W^f/W^f* mice (17). This suggests that the release of histamine is

downstream of UV-induced mast cell activation. While the trigger for histamine release by mast cells is not entirely clear, it appears that direct exposure to UV (especially the deeper penetrating, longer wavelength, UVA) (88) or possibly UV-induced platelet-activating factor (PAF) (89) triggers histamine release. Mast cell-derived histamine stimulates keratinocyte production of PGE₂ that in turn precipitates the simultaneous upregulation of IL-10 and downregulation of IL-12 (90). Together with histamine itself, these molecular events can alter the function of cutaneous DC such that they adopt an immune suppressive phenotype. For example, mast cell-derived histamine has been shown to suppress the development of robust, Th1-mediated anti-tumour immune responses (91) by altering the antigen-presenting capacity of DC (92). Histamine may also influence the migration of inflammatory cells both into and away from the UV-irradiated site (93). Meanwhile, in skin-draining lymph nodes where tumour-specific adaptive immune cells are activated, histamine regulates the proliferation and cytokine profile of T lymphocytes (94). Hence, UV-induced histamine release by mast cells is likely to have a significant impact on the ability of the host anti-tumour immune response to destroy skin cancers.

Interleukin-10

IL-10 is a well-known anti-inflammatory cytokine that acts to suppress Th1-anti-tumour immune responses (95). As already discussed, IL-10 is significantly upregulated by UV exposure and is required for both UV-induced immune suppression (96) and skin tumour development (67). Because keratinocytes (97) and skin-infiltrating macrophages (98) are important sources of UV-induced IL-10, it remains to be determined whether mast cells are also a critical source of this immunomodulatory cytokine. It has only recently become clear that a number of UV-induced inflammatory mediators (e.g. vitamin D₃ and IL-33) trigger the production of IL-10 by mast cells. This is likely to be important for the growth of skin tumours because high levels of IL-10 production are associated with tumour progression of both melanoma (99) and non-melanoma skin cancer (100). The precise mechanisms by which high levels of UV-induced IL-10 could enhance skin cancer growth are unclear. In the presence of high amounts of IL-10, DC develop a tolerogenic phenotype (101) that induces anergy in melanoma-specific T cells (102). In murine models of melanoma, the induction of IL-10 at tumour sites suppresses anti-tumour immunity by activating immunosuppressive CD4⁺ T regs (103). Alternatively, IL-10 may suppress macrophage function or even directly enhance melanoma cell proliferation and/or angiogenesis (104). Yet another possibility is a role for mast cell-derived IL-10 in the promotion of endothelial progenitor cell survival and function as has been recently demonstrated in a model of myocardial infarction (105). Hence, UV-induced IL-10, by keratinocytes, macrophages or mast cells, at tumour sites is likely to contribute towards the generation of an immunosuppressive microenvironment favourable for tumour growth.

Tumour necrosis factor (TNF)

Mast cells are recognized as a major source of TNF, a key soluble mediator in UV-induced local immunosuppression. High levels of TNF have been reported in UV-exposed skin. TNF is thought to be the main effector molecule that induces LC to migrate to the skin-draining lymph nodes. While subcutaneous administration of TNF has been shown to promote morphological changes in LCs

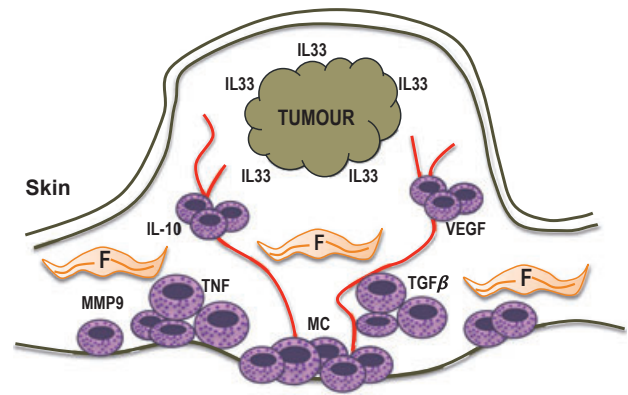


Figure 4. Role of mast cells in UV-induced skin tumour initiation and progression. UV irradiation stimulates mast cell recruitment and activation in the skin. Dermal mast cells are then triggered to release their inflammatory mediators (Table 1) that act to suppress the anti-tumour immune response (Fig. 2). Following exposure to chronic doses of UV, mast cells initiate skin tumour development by promoting angiogenesis (117,142), vasculogenesis (167) and tissue remodelling (117). During the early growth of skin tumours, mast cells rapidly infiltrate the surrounding tumour tissue (usually at the boundary between healthy tissue and the tumour tissues). Within this tumour microenvironment, mast cells release a range of mediators that promote tumour growth and survival including IL-10 (67), MMP-9 (117,142,167), TNF (107,109), VEGF (118), TGF- β (118), as well as the conversion of dermal fibroblasts (F) into tumour-promoting α -SMA-expressing myofibroblasts (151).

(106), treatment with antibodies against TNF protected LCs from such alterations (106,107). In chemical skin carcinogenesis studies, TNF-deficient mice were resistant to the development of benign and malignant skin tumours (108). This lack of TNF affected skin inflammation as lower levels of neutrophil infiltration were observed in the skin of TNF-deficient mice (108). Therefore, TNF could potentially influence tumour and stromal cells during tumour development (Fig. 4). This may also be relevant to UV-induced skin tumours because UV triggers the release of TNF from dermal mast cells (107,109). Mast cell-derived TNF induces epidermal LC migration to the skin-draining lymph nodes (110). This is likely to be an important component of the ability of UV to suppress immune responses because LCs migrating from UV-exposed skin have been shown to mediate the activation of IL-4-producing regulatory natural killer (NK)-T cells in draining lymph nodes (111). Very recently, Artuc et al. (112) showed in this journal that mast cell-derived TNF (together with histamine) may modulate the anti-tumour immune response by inducing the production and release of IL-6 and IL-8 in human melanoma and non-melanoma skin tumours.

Consequences of UV-induced mast cell recruitment and activation on tumour growth

Vascular endothelial growth factor

Tumours rely on a continual vascular supply for their growth as otherwise their growth is severely limited. UV triggers an angiogenic switch in the skin by simultaneously upregulating the pro-angiogenic vascular endothelial growth factor (VEGF) (113) and downregulating the anti-angiogenic factor thrombospondin-1 (114). While keratinocytes appear to be an important source of UV-induced VEGF, expression is also observed deeper in the dermis (113). Mast cells also produce VEGF (115), and VEGF-producing mast cells have been observed in human BCC (116). Indeed, mast cells play an important pro-angiogenic role during skin carcinogenesis (117), although whether UV is able to trigger VEGF release from dermal mast cells is not yet known. UV-induced

inflammatory mediators, which may promote VEGF production by mast cells, include endothelin-1 (ET-1) (118) and PGE₂ (119). Both of these are upregulated in UV-exposed skin (120,121). More recently it was demonstrated that IL-33 acts synergistically with SP to enhance VEGF secretion by mast cells (76). This leads us to hypothesize that another possible mechanism by which UV upregulates VEGF in the skin is via UV-induced IL-33 (77) and SP (76). We have recently demonstrated that UV-induced skin tumours can also produce IL-33 even in the absence of further UV exposure (77). Therefore, established UV-induced skin tumours may facilitate their own growth via an IL-33-mast cell-mediated mechanism (Fig. 4). This pro-angiogenic axis may be further amplified by VEGF-mediated recruitment of more mast cells to the irradiated skin and/or UV-induced skin tumour (122).

Transforming growth factor (TGF) β

A significant increase in TGF β within human dermis is observed 24–72 h after exposure to solar simulated UV (123). Although TGF β acts as a tumour suppressor during the early stages of skin tumour development (124), it can also have tumour-promoting effects. For example, TGF β stimulates skin tumour invasion and metastasis by initiating epithelial to mesenchymal transition in keratinocytes (124). TGF β can also promote tumour growth and metastasis by inducing fibroblast production of matrix metalloproteinase-9 (MMP-9; more on this later) (125). Mast cells are an important source of TGF β (126,127). Mast cell production of TGF β has been shown to be involved in the induction of collagen gene expression in dermal fibroblasts (126). In this way, mast cells contribute to dermal fibrosis, which lays the foundation for a pro-tumorigenic microenvironment. Hence, UV induction of TGF β is likely to have a significant effect on skin tumour growth and metastasis (for a recent review see ref. (128)). TGF β is also likely to influence the success of anti-tumour immune responses as it suppresses anti-tumour immunity. For example, mast cell-derived TGF β significantly impacts on the development of FoxP3⁺ regulatory T cells (129) that are found infiltrating malignant melanoma (130,131). It has recently been reported in this journal that the presence of high numbers of FoxP3⁺ T regs may have important diagnostic implications for human melanoma patients (132). TGF β also affects the recruitment and differentiation of tumour-associated neutrophils (TANs) (133) and suppressor macrophages (TAMs) in UV-induced skin tumours (134). TGF β further facilitates skin tumour escape from the host anti-tumour immune response by inhibiting the migration of DC to tumour draining lymph nodes (135), although whether this TGF β originates from the tumour cells themselves or dermal mast cells or both remains to be determined. TGF β mediates the recruitment of more immunomodulatory mast cells to the irradiated dermis and/or UV-induced skin tumour, further amplifying its effects (122). Mast cells can be induced to produce TGF β in response to UV-inflammatory mediators such as ET-1 (118), while reactive oxygen species produced after UV exposure induce TGF β synthesis and activation (136). A role for *cis*-UCA in UV-induced TGF β has been ruled out (137), although it remains to be determined whether other UV-induced inflammatory mediators (such as PGE₂ or IL-33) contribute to mast cell production of TGF β .

Matrix metalloproteinase (MMP)-9

Matrix metalloproteinase-9 (MMP-9) is one of a family of zinc-requiring proteases capable of degrading extracellular matrix

components [for a recent review see ref. (138)]. Because of the abundance of extracellular matrix components in skin, MMPs play a particularly important role in skin tumour progression (139). MMP-9 in particular is heavily implicated in both the degradation of extracellular matrix in UV-irradiated skin, which ultimately leads to wrinkle formation (140,141), and skin cancer (142). A role for MMP-9 in cellular migration is also documented, with a requirement for MMP-9 in the migration of DC from the skin to the draining lymph nodes (143). Mast cells produce MMP-9 (144), implicating them in the modulation of inflammatory cell migration into and away from the UV-irradiated skin, as well as more generally in the skin carcinogenic process by influencing tumour cell growth, survival, invasion and metastasis (Fig. 4) (142,145). However, it still remains to be determined whether the cellular source of UV-induced MMP-9 is mast cell derived.

The tumour stroma

It is now clear that the surrounding stroma or microenvironment supports tumour growth and facilitates immune evasion and metastasis (for a recent review see (146)). A role for mast cells in manipulating the tumour stroma has emerged (19), and some of the factors involved (e.g. VEGF and MMP-9) have already been discussed. Tumour stromal fibroblasts or carcinoma-associated fibroblasts promote tumour growth and angiogenesis via production of the endothelial progenitor cell chemoattractant, CXCL12 (147). This is relevant because we have shown that CXCL12 is also a potent mast cell chemoattractant following exposure to UV (37). In the skin, close contact and collaboration between dermal mast cells and fibroblasts conspire to create a microenvironment permissive for skin tumour establishment, growth and eventually metastasis. For example, mast cell-fibroblast interactions promote the production of MMP-9 (148), and we have shown that UV-induced IL-33 from fibroblasts has the potential to act on adjacent dermal mast cells to suppress anti-tumour immune responses (77).

A hallmark of tumour-associated fibroblasts is their differentiation into myofibroblasts which can be seen by their upregulated expression of α -smooth muscle actin (α -SMA) (149). In the skin, myofibroblasts play a critical role in wound healing but are not derived from circulating progenitors (150), implying that there is a signal that switches resident fibroblasts to a myofibroblast phenotype. Mast cell-derived tryptase plays a critical role in providing this differentiation signal (151,152), and mast cell production of TGF β (126,127) may also be involved because TGF β is well known for its role in the induction of myofibroblasts, especially in the dermis (153). Other UV-induced mediators that could be involved include NGF (154) and ET-1 (155) (discussed above), both of which can induce dermal fibroblast transformation into α -SMA-expressing myofibroblasts. More recently, it was shown that bone marrow-derived Sca-1⁺c-KIT⁺ cells activated stromal fibroblasts to become α -SMA⁺ myofibroblasts by producing granulatin (156). It remains to be determined whether UV-activated dermal mast cells can produce granulatin. Whether UV-exposed skin contains myofibroblasts also remains to be determined; however, UV-induced skin cancers such as squamous- (157) and basal-cell carcinoma (158) as well as melanoma (159) are all surrounded by α -SMA⁺ myofibroblasts. This is likely to have a profound effect on skin tumour initiation, growth and metastasis because myofibroblasts collaborate with mast cells as well as the tumours themselves

to produce significant amounts of TGF β , CXCL12, MMPs, VEGF in a self-perpetuating pro-tumorigenic loop (160).

Conclusions

Excessive exposure to the UV component of sunlight leads to skin cancer. It is clear that mast cells play a key role throughout this carcinogenic process. Initially, UV recruits mast cells to the skin, activating them to release their immune suppressive inflammatory mediators. UV also triggers mast cell production of tissue remodelling and pro-angiogenic factors that facilitate tumour growth, survival and differentiation. The cellular and molecular mechanisms by which UV modulates the recruitment and activation of dermal mast cells, therefore, represent attractive therapeutic targets for the prevention and treatment of UV-induced skin cancer. Despite the recent advances that have been discussed here, further research is still required to elucidate the role played by mast cells in skin carcinogenesis. Functional experiments utilizing new mast cell-deficient animal models (161) are likely to clarify their role in this context.

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Conflict of interests

The authors have declared no conflicting interests.

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