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The Role of Cytokines and Chemokines in the Development of Basal Cell Carcinoma

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1. Introduction

The immune system plays an important role in surveillance against tumor development, and it is widely known that cancer cells protect themselves against the host's anti-tumor immune defense. Cancer cells have several means of evading the antitumor immunity, one of which is the production of immune modulators such as cytokines and chemokines. These factors can either promote or block immune responses. Many of these molecules are used by cancer cells to promote tumor progression including cell proliferation, cell migration, matrix remodeling, immune suppression and angiogenesis. On the other hand, some molecules are involved in immunotherapeutics for the purpose of enhancing and modifying antitumor immune responses.

In basal cell carcinoma (BCC), several cytokines and chemokines and their receptors are associated with the development of this cutaneous cancer. There are varying degrees of inflammation in BCC. The majority of peritumor inflammatory cells are lymphocytes and most are T cells (1). It has been proposed that the tumor microenvironment of BCC is generally Th2 dominant. T regulatory cells and immature dendritic cells mediated by Th2 cytokines cause immunosuppression and decreased immunity to BCC (2). Tumor-associated macrophages (TAM), which are polarized to M2 type, are associated with tumor invasion and angiogenesis in BCC (3). In this chapter, we focus on cytokines and chemokines which may influence and enhance these immunosuppressive networks.

2. IL-6

Interleukin-6 (IL-6) is a pro-inflammatory cytokine, which can induce tumor progression by manipulating immune responses in the tumor microenvironment. IL-6 is directly related to epidermal hyperproliferation in psoriasis (4). There are many experimental evidences that IL-6 is associated with BCC. Overexpression of IL-6 in BCC cell lines increases anti-apoptotic activity and tumorigenic potency (5). The phosphotidyl inositol 3-kinase (PI3K)/Akt signal pathway is involved in such anti-apoptosis (6). On the other hand, IL-6 induces bFGF-dependent angiogenesis in BCC cell line via JAK/STAT3 and PI3k/Akt pathways (7). IL-6 is also involved in CXCL-12 (SDF-1)-enhanced angiogenesis via activating ERK1/2 and NF-κB (8). IL-6 expression is associated with a significant increase of IL-8 (CXCL8) expression in

BCC (9, 10), one of which functions is tumor angiogenesis. The expression of these two cytokines shows a significant positive correlation (10).

A single nucleotide polymorphism (SNP) in the promoter regions of IL-6 gene (*IL6*) is associated with the risk of BCC. The promoter region of *IL6* contains several SNPs, including –634G>C, –597G>A and –174G>C. It has been reported that *IL6* –597 G>A is significantly associated with BCC risk (11). However, others reported that there was no difference for genotype distributions of SNPs in the promoter region of *IL6* between the BCC cases and controls, while linkage disequilibrium was observed between the –174 and –597 alleles in the *IL6* (12).

3. IL-10

Interleukin-10 (IL-10) is a major immunosuppressive cytokine that plays a critical regulatory role in several areas of the immune system. It contributes to immunosuppression in the tumor microenvironment and may render it permissive for infiltration of cancer cells. IL-10 is upregulated in both melanoma (13-15) and non-melanoma skin cancer including BCC (2, 16, 17). The presence of IL-10 in BCC is associated with the lack of expression of HLA-DR, ICAM-1, CD40 and CD80 and the inconsistent expression of HLA-ABC in BCC (17). BCC is regarded as an indolent (slow growing) cancer with limited metastatic potential. While IL-10 expression by melanoma cells correlates with melanoma progression and development of metastatic competence (18), there is no clear correlation between IL-10 expression and tumor invasiveness of BCC. IL-10 can be detected by both aggressive BCC and nonaggressive BCC such as superficial BCC. However, there are discrepant results regarding IL-10 expression in superficial BCC. Urosevic *et al*. found that superficial BCC cells were uniformly negative for IL-10 expression at baseline and showed little change after imiquimod treatment (19).

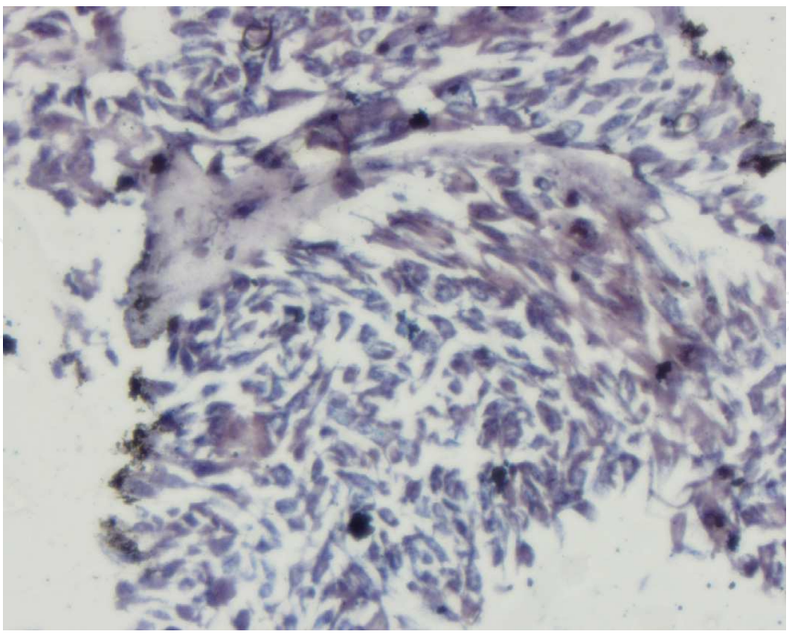


Fig. 1. IL-10 mRNA is expressed by tumor cells in basal cell carcinoma (blue/purple color, RT *in situ* PCR).

SNP in the promoter regions of IL-10 gene (*IL10*) is associated with the risk of BCC. *IL10* –1082G>A is detected in BCC (12), and this polymorphism, as well as tumor necrosis factor-alpha (TNF-α) gene *TNF* –308G>A polymorphism, is more prevalent in aggressive BCC (20). However, others reported that there was no significant association between BCC and *IL10* –1082 (11).

4. CXCL12 and CXCR4

CXCL12 (SDF-1) is a proinflammatory chemokine produced in response to inflammatory stimuli. This chemokine functions as a chemoattractant for lymphocytes. CXCL12 also plays an important role in tumor angiogenesis through binding to its receptor, CXCR4. CXCR4 expression enhances tumorigenesis and angiogenesis in BCC. Such CXCL12-enhanced angiogenesis involves the ERK1/2 and NF-*κ*B pathways mediated by IL-6 (8). CXCR4 is especially expressed in noduloulcerative and sclerosing types of BCC and is associated with more aggressive behavior (21). CXCL12 directs BCC invasion by upregulating gelatinase activity of matrix metalloproteinase-13 (MMP-13). The transcriptional regulation of MMP-13 by CXCL12 is mediated by phosphorylation of ERK1/2 and c-Jun/AP-1 activation (22). CXCL12 also upregulates several angiogenesis-associated genes including interferon alpha-inducible protein 27 (IFI27), bone morphogenetic protein 6 (BMP6) and cyclooxygenase 2 (COX-2) (8).

5. CXCL9, CXCL10, CXCL11 and CXCR3

CXCL9 (MIG), CXCL10 (IP-10) and CXCL11 (I-TAC) are chemokines that are induced by interferon during inflammatory responses. These chemokines bind to a common receptor, CXCR3. They can promote chemotaxis of activated T cells and NK cells through binding to CXCR3. The most recent attention has been given to the role of these chemokines in tumorigenesis of BCC. It has been reported that CXCL9, CXCL10, CXCL11, and their receptor CXCR3 are significantly upregulated in BCC. CXCR3, CXCL10, and CXCL11, but not CXCL9, colocalize with keratin 17, which is a BCC keratinocyte marker. Exposure of BCC cells to CXCL11 *in vitro* enhances keratinocyte cell proliferation (23). CXCL9, CXCL10 and CXCL11 promote expression of functional indoleamine 2,3-dioxygenase (IDO), which also colocalizes with keratin 17 (24). Thus, CXCR3 and its ligands may be important in tumorigenesis of BCC.

6. IL-8 (CXCL8)

Interleukin-8 (IL-8, CXCL8) is a chemokine produced by inflammatory cells and other cell types. This chemokine is one of the major mediators of the inflammatory response. It functions as a chemoattractant, but is also known as an angiogenic factor. IL-8 is associated with tumor angiogenesis in many solid tumors. It has been reported IL-8 is highly expressed in BCC (25). As described earlier in this chapter, IL-8 expression is associated with a significant increase of IL-6 expression in BCC (9, 10), and is positively correlated with IL-6 expression (10). However, the detailed mechanisms of IL-8 involved in the development of BCC are not fully understood.

7. CCL27

CCL27 is a chemokine that functions as a chemoattractant by interacting with its receptor, CCR10. This chemokine regulates T cell homing under homeostatic and inflammatory

conditions, and plays a role in T cell-mediated inflammation of the skin. In BCC, the downregulating of CCL27 expression is associated with tumor immune escape. A significant decrease in CCL27 expression is also observed in squamous cell carcinoma and actinic keratosis. These skin tumors may evade T cell-mediated antitumor immune responses by down-regulating the expression of CCL27 through the activation of epidermal growth factor receptor (EGFR)-Ras-MAPK-signaling pathways (26).

8. IFN-γ

Interferon-gamma (IFN-γ) is a cytokine that is critical for immune responses against cancer. IFN-γ binding to the receptor activates the JAK-STAT pathway. In BCC, The expression of IFN-γ receptor is significantly decreased on the cancer cells compared with the overlying epidermis. The absence or paucity of IFN-γ receptor and the absence of intercellular adhesion molecule-1 (ICAM-1) may explain the lack of tumor-infiltrating cells and the lack of an active cell-mediated immune response in BCC (27).

On the other hand, Th1 cytokines including IFN-γ play a role in spontaneously regressing BCC. Some cases of BCC may show spontaneous regression in the absence of therapy. Such spontaneous regression is mediated by activated CD4+ T cells, and IFN-γ is elevated in actively regressing BCC (28). There is a significantly increased number of CD4+ T cells infiltrating regressing tumors, and the expression of IL-2 receptor, which is an early activation marker for T cells is also increased (29). Abundant CD8+ T cells and interferon signal transduction is associated with partial host antitumor response (2).

Imiquimod has been shown to be efficacious as a topical treatment for BCC. Imiquimod is a Toll-like receptor 7 (TLR7) agonist, which induces interferon and other cytokines through the immune system and stimulates innate and adaptive cell-mediated immunity. Clinical studies have demonstrated clinical and histological clearance of superficial BCC after treatment with imiquimod 5% cream (30-32). Imiquimod treatment is associated with the early appearance of lymphocytes and macrophages. This early response tends to be a mixed cellular response of CD4 cells, activated dendritic cells and macrophages, with later infiltration by CD8 T cells (33). Application of imiquimod induces a cascade of Th1 cytokines including IFN-α, TNF-α, IL-1α, IL-12, and IFN-γ, with profound effects on innate and adaptive immunity and on immunologic memory and antigen presentation. IFN-γ is produced by CD4 and CD8 T cells. IFN-γ is associated with the enhanced expression of ICAM-1, promoting the influx of immune cells. Imiquimod treatment also induces a massive increase in macrophage peritumoral and intratumoral infiltration (19). Thus, the TLR7-agonist plays an important role in inducing a lymphocytic infiltrate by promoting specific Th1 cellular immune response capable of eliminating cancer cells (34).

9. FasL (CD95L) and Fas (CD95)

Fas ligand (FasL, CD95L) belongs to the tumor necrosis factor (TNF) family. FasL binds to its receptor, Fas (CD95), and induces apoptosis. Apoptosis via FasL/Fas pathway plays an important role in the regulation of the immune system. FasL expressed by cacncer cells induces apoptosis of infiltrating lymphocytes and they can evade immune surveillance, contributing to cancer progression. BCC has been reported to lack Fas expression (19, 35),

while they commonly retain the expression of FasL (36). In normal skin, Fas is expressed by keratinocytes in the basal layer. Fas expression is up-regulated in chronically sun-damaged skin. Actinic keratosis does not express Fas. Squamous cell carcinoma focally expresses Fas at the sites of contact with lymphocytes (35).

It has been suggested that BCC can evade host immune surveillance by expressing FasL (37). However, different results were obtained for the FasL expression in BCC (19, 38, 39), and the issue of FasL expression in BCC is still debatable. After imiquimod treatment, the infiltrating cells demonstrate an increase in Fas/FasL expression, while Fas expression by BCC cells remains unaffected and FasL expression demonstrates either an increase or a decrease in different cases (19). After intralesional IFN-α treatment, BCC cells become Fas-positive with signs of tumor regression as a result of tumor cell apoptosis (36). Thus, Fas/FasL pathway may be associated with tumor regression by such treatments.

Substance	**Alternative name**	**Receptor**	**Action**
IL-6		IL6R (CD126)	Anti-apoptotic activity
IL-8	CXCL8	IL8RA (CXCR1, CD181); IL8RB (CXCR2, CD182)	Tumor angiogenesis
IL-10		IL10RA (CDw210a); IL10RB (CDw210b)	Immune suppression
CXCL9	MIG	CXCR3 (CD183)	Tumorigenesis
CXCL10	IP-10	CXCR3 (CD183)	Tumorigenesis
CXCL11	I-TAC	CXCR3 (CD183)	Tumorigenesis
CXCL12	SDF-1	CXCR4 (CD184)	Tumor angiogenesis; stromal invasion
CCL27	CTACK, ILC, ESKine	CCR10	Immune escape by down-regulation of CCL27
IFN-γ		IFNGR1 (CD119); IFNGR2	Tumor regression; immune escape by down-regulation of IFN-γ receptor
FasL	CD95L	Fas (CD95)	Immune escape

Table 1. Cytokines and chemokines in the pathogenesis of Basal Cell Carcinoma.

10. Conclusions

There is much more work to be done in order to adequately characterize the clinical significance of cytokines, chemokines and related molecules in BCC. Studies thus far show that the factors described in this chapter play an integral role in BCC development and immunosuppression. A better understanding of these interactions may facilitate development of more potent immune-based treatment for BCC.

11. References

[1] Miller SJ. Biology of basal cell carcinoma (Part II). J Am Acad Dermatol 1991;24:161-175.

[2] Kaporis HG, Guttman-Yassky E, Lowes MA, *et al.* Human basal cell carcinoma is associated with Foxp3+ T cells in a Th2 dominant microenvironment. J Invest Dermatol 2007;127:2391-2398.

[3] Tjiu JW, Chen JS, Shun CT, *et al.* Tumor-associated macrophage-induced invasion and angiogenesis of human basal cell carcinoma cells by cyclooxygenase-2 induction. J Invest Dermatol 2009;129:1016-1025.

[4] Neuner P, Urbanski A, Trautinger F, *et al.* Increased IL-6 production by monocytes and keratinocytes in patients with psoriasis. J Invest Dermatol 1991;97:27-33.

[5] Jee SH, Shen SC, Chiu HC, Tsai WL, Kuo ML. Overexpression of interleukin-6 in human basal cell carcinoma cell lines increases anti-apoptotic activity and tumorigenic potency. Oncogene 2001;20:198-208.

[6] Jee SH, Chiu HC, Tsai TF, *et al.* The phosphotidyl inositol 3-kinase/Akt signal pathway is involved in interleukin-6-mediated Mcl-1 upregulation and anti-apoptosis activity in basal cell carcinoma cells. J Invest Dermatol 2002;119:1121-1127.

[7] Jee SH, Chu CY, Chiu HC, *et al.* Interleukin-6 induced basic fibroblast growth factor-dependent angiogenesis in basal cell carcinoma cell line via JAK/STAT3 and PI3-kinase/Akt pathways. J Invest Dermatol 2004;123:1169-1175.

[8] Chu CY, Cha ST, Lin WC, *et al.* Stromal cell-derived factor-1α (SDF-1α/CXCL12)-enhanced angiogenesis of human basal cell carcinoma cells involves ERK1/2–NF-κB/interleukin-6 pathway. Carcinogenesis 2009;30:205-213.

[9] Yen HT, Chiang LC, Wen KH, *et al.* The expression of cytokines by an established basal cell carcinoma cell line (BCC-1/KMC) compared with cultured normal keratinocytes. Arch Dermatol Res 1996;288:157-161.

[10] Gambichler T, Skrygan M, Hyun J, *et al.* Cytokine mRNA expression in basal cell carcinoma. Arch Dermatol Res 2006;298:139-141.

[11] Wilkening S, Hemminki K, Rudnai P, *et al.* Case-control study in basal cell carcinoma of the skin: single nucleotide polymorphisms in three interleukin promoters pre-analysed in pooled DNA. Br J Dermatol 2006;155:1139-1144.

[12] Festa F, Kumar R, Sanyal S, *et al.* Basal cell carcinoma and variants in genes coding for immune response, DNA repair, folate and iron metabolism. Mutat Res 2005;574:105-111.

[13] Chen Q, Daniel V, Maher DW, Hersey P. Production of IL-10 by melanoma cells: examination of its role in immunosuppression mediated by melanoma. Int J Cancer 1994;56:755-760.

[14] Krüger-Krasagakes S, Krasagakis K, Garbe C, *et al.* Expression of interleukin 10 in human melanoma. Br J Cancer 1994;70:1182-1185.

[15] Gerlini G, Tun-Kyi A, Dudli C, *et al.* Metastatic melanoma secreted IL-10 down-regulates CD1 molecules on dendritic cells in metastatic tumor lesions. Am J Pathol 2004;165:1853-1863.

[16] Kim J, Modlin RL, Moy RL, *et al.* IL-10 production in cutaneous basal and squamous cell carcinomas. A mechanism for evading the local T cell immune response. J Immunol 1995;155:2240-2247.

[17] Kooy AJ, Prens EP, Van Heukelum A, *et al.* Interferon-gamma-induced ICAM-1 and CD40 expression, complete lack of HLA-DR and CD80 (B7.1), and inconsistent HLA-ABC expression in basal cell carcinoma: a possible role for interleukin-10? J Pathol 1999;187:351-357.

[18] Itakura E, Huang RR, Wen DR, *et al.* IL-10 expression by primary tumor cells correlates with melanoma progression from radial to vertical growth phase and development of metastatic competence. Mod Pathol 2011;24:801-809.

[19] Urosevic M, Maier T, Benninghoff B, *et al.* Mechanisms underlying imiquimod-induced regression of basal cell carcinoma in vivo. Arch Dermatol 2003;139:1325-1332.

[20] Fernandes H, Fernandes N, Bhattacharya S, *et al.* Molecular signatures linked with aggressive behavior in Basal cell carcinoma: a report of 6 cases. Am J Dermatopathol 2010;32:550-556.

[21] Chen GS, Yu HS, Lan CC, *et al.* CXC chemokine receptor CXCR4 expression enhances tumorigenesis and angiogenesis of basal cell carcinoma. Br J Dermatol 2006;154:910-918.

[22] Chu CY, Cha ST, Chang CC, *et al.* Involvement of matrix metalloproteinase-13 in stromal-cell-derived factor 1α-directed invasion of human basal cell carcinoma cells. Oncogene 2007;26:2491-2501.

[23] Lo BKK, Yu M, Zloty D, *et al.* CXCR3/ligands are significantly involved in the tumorigenesis of basal cell carcinomas. Am J Pathol 2010;176:2435-2446.

[24] Lo BKK, Jalili RB, Zloty D, *et al.* CXCR3 ligands promote expression of functional indoleamine 2,3-dioxygenase (IDO) in basal cell carcinoma keratinocytes. Br J Dermatol 2011:Epub.

[25] Szepietowski JC, Walker C, McKenna DB, Hunter JA, McKenzie RC. Leukaemia inhibitory factor and interleukin-8 expression in nonmelanoma skin cancers. Clin Exp Dermatol 2001;26:72-78.

[26] Pivarcsi A, Muller A, Hippe A, *et al.* Tumor immune escape by the loss of homeostatic chemokine expression. Proc Natl Acad Sci U S A 2007;104:19055-19060.

[27] Kooy AJ, Tank B, Vuzevski VD, van Joost T, Prens EP. Expression of interferon-gamma receptors and interferon-gamma-induced up-regulation of intercellular adhesion molecule-1 in basal cell carcinoma; decreased expression of IFN-γR and shedding of ICAM-1 as a means to escape immune surveillance. J Pathol 1998;184:169-176.

[28] Wong DA, Bishop GA, Lowes MA, *et al.* Cytokine profiles in spontaneously regressing basal cell carcinomas. Br J Dermatol 2000;143:91-98.

[29] Hunt MJ, Halliday GM, Weedon D, Cooke BE, Barnetson RS. Regression in basal cell carcinoma: an immunohistochemical analysis. Brit J Dermatol 1994;130:1-8.

[30] Geisse J, Caro I, Lindholm J, *et al.* Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from two phase III, randomized, vehicle-controlled studies. J Am Acad Dermatol 2004;50:722-733.

[31] Beutner KR, Geisse JK, Helman D, *et al.* Therapeutic response of basal cell carcinoma to the immune response modifier imiquimod 5% cream. J Am Acad Dermatol 1999;41:1002-1007.

[32] Schulze HJ, Cribier B, Requena L, *et al.* Imiquimod 5% cream for the treatment of superficial basal cell carcinoma: results from a randomized vehicle-controlled phase III study in Europe. Br J Dermatol 2005;152:939-947.

[33] Barnetson RS, Satchell A, Zhuang L, Slade HB, Halliday GM. Imiquimod induced regression of clinically diagnosed superficial basal cell carcinoma is associated with early infiltration by CD4 T cells and dendritic cells. Clin Exp Dermatol 2004;29:639-643.

[34] Wenzel J, Uerlich M, Haller O, Bieber T, Tueting T. Enhanced type I interferon signaling and recruitment of chemokine receptor CXCR3-expressing lymphocytes into the skin following treatment with the TLR7-agonist imiquimod. J Cutan Pathol 2005;32:257-262.

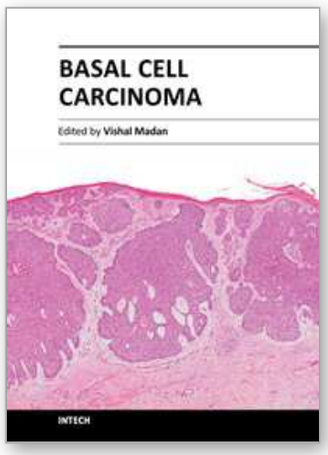
[35] Filipowicz E, Adegboyega P, Sanchez RL, Gatalica Z. Expression of CD95 (Fas) in sun-exposed human skin and cutaneous carcinomas. Cancer 2002;94:814-819.

[36] Buechner SA, Wernli M, Harr T, *et al.* Regression of basal cell carcinoma by intralesional interferon-alpha treatment is mediated by CD95 (Apo-1/Fas)–CD95 ligand–induced suicide. J Clin Invest 1997;100:2691-2696.

[37] Ji J, Wernli M, Mielgo A, Buechner SA, Erb P. Fas-ligand gene silencing in basal cell carcinoma tissue with small interfering RNA. Gene Ther 2005;12:678-684.

[38] Gutierrez-Steil C, Wrone-Smith T, Sun X, *et al.* Sunlight-induced basal cell carcinoma tumor cells and ultraviolet-B-irradiated psoriatic plaques express Fas ligand (CD95L). J Clin Invest 1998;101:33-39.

[39] Lee SH, Jang JJ, Lee JY, *et al.* Fas ligand is expressed in normal skin and in some cutaneous malignancies. Br J Dermatol 1998;139:186-191.



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Basal cell carcinoma is the commonest cutaneous malignancy. The last decade has witnessed exponential research which has broadened our understanding of the pathogenesis of basal cell carcinomas. This is also important from a therapeutic point of view as targeted approach to therapy is now being increasingly experimented. Although it is impossible to condense and present all good research in one book, the authors have to be commended on presenting their research on several aspects of basal cell carcinoma in a succinct manner, which shall not only enhance our understanding of, but also hopefully via this open exchange of ideas pave ways for successful targeted therapy of the commonest human cancer.

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