International Journal of Basic and Applied Medical Sciences ISSN: 2277-2103 (Online)
An Online International Journal Available at http://www.cibtech.org/jms.htm
2012 Vol. 2 (2) May-August, pp102-107/Yadav et al.
Review Article

ROLE OF CYTOKINE SIGNALLING IN HEAD AND NECK CANCERS

Navneet Kumar Yadav[1], Pooja Shukla[1], Poonam Singh[1], F.W. Bansode[2] and *R.K.Singh[1]
[1]Division of Toxicology and [2]Endocrinology
CSIR- CDRI Chattar Manzil Palace Lucknow-226001
**Author for Correspondence*

ABSTRACT
Head and neck squamous cell carcinoma (HNSCC) is the 6th most common type of malignancy worldwide. Cytokines are molecules that influence activation, growth, and differentiation of several target cells, Cells of head and neck squamous cell carcinoma (HNSCC) develop molecular strategies in order to evade growth inhibitory effects of cytokines present in the tumor microenvironment and malignant transformation. Cytokine-driven immunotherapy is a nonspecific, passive approach aiming to augment or boost immune cells with antitumor activity.

INTRODUCTION
Head and neck squamous cell carcinoma (HNSCC) is the 6th most common type of malignancy worldwide, and represents over 6% of the global cancer burden. HNSCC accounts for nearly 650,000 new cases of cancer worldwide, and over 35,000 deaths each year. Ninety percent cancers in the head and neck area are squamous cell cancers (HNSCC) from mucosal epithelium Sengupta (2012). Historically, HNSCC has been a challenging disease to manage, with locally advanced disease often requiring a multidisciplinary approach of surgery, chemotherapy and radiation Walker *et al.,* (2012).

Cytokines are molecules that influence activation, growth, and differentiation of several target cells, and more than 100 types of cytokines have been identified. They are produced by different types of cells, exhibiting less restricted tissue specificity than hormones Franco Lumachi *et al.*, (2010). They are a heterogeneous group of soluble small polypeptides or glycoproteins, which exert pleiotropic and redundant effects that promote growth, differentiation and activation of normal cells. Cytokines can have either pro- or anti-inflammatory activity or immunosuppressive activity, depending on the microenvironment. Immune cells are the major source of cytokines but many human cells are capable of producing them and, importantly, their production acts as a means of communication between both cells and tissues Borish *et al.,* (2003).

Cells of head and neck squamous cell carcinoma (HNSCC) develop molecular strategies in order to evade growth inhibitory effects of cytokines present in the tumor microenvironment. Therefore, the malignant transformation process is strongly associated with an altered response to cytokine stimulation. Douglas WG *et al.*, (2004). Prominent HNSCC-derived cytokines are interleukin-4 (IL-4), IL-6, IL-8, IL- 10, granulocyte macrophage-colony-stimulating factor (GM-CSF), vascular endothelial growth factor (VEGF), prostaglandin E2 (PGE2) as well as basic fibroblast growth factor (bFGF) Chen *et al*., (1999); Woods *et al.,* (1998); Eisma *et al*., (1997). HNSCC relevant cytokines and their functions are summarized in Table 1.

Decreasing cytokine and growth factor levels in serum are associated with Response to therapy, while increasing levels are related to cancer progression and recurrence. IL-6 increases VEGF expression and the invasive potential in cell lines Noda (2007), and its expression correlates with poor prognosis in HNSCC patients Duffy *et al.,* (2008).IL-6 involve in mediating Epithelial–mesenchymal transition in head and neck tumor cells and increasing their metastatic potential Yadav *et al.,* (2011)

IL-8 and GRO1 serve as chemoattractants for neutrophils, monocytes, and endothelial cells, which are all major constituents of the inflammatory and angiogenesis response, and their expression promotes aggressive growth and metastasis Van (2007). In addition, IL-1 and IL-6 are potent inducers of HGF production by stromal cells, such as fibroblasts, and HGF is capable of further enhancing IL-8 and VEGF expression Worden (2005) DiNatale *et al.,* (2011) .Several cytokines and growth factors also activate signal pathways that promote the malignant phenotype. TNFa, IL-1, HGF, and their receptors promote

activation of the mitogen activated protein kinase-activator protein-1 (MAPK-AP-1), nuclear factor-kappa B (NF-kB), and phosphotidylinositol-3 kinase (PI3K)/Akt pathways. Umemura *et al.***,** (2012) Luangdilok *et al*., (2011**)** Amornphimoltham *et al.,* (2011). Epidermal growth factor (EGF) and IL-6 activate signal transducer and activating transcription factor-3 (STAT3) in HNSCC cells Leeman *et al*.,(2006), Lee *et al*.,(2008). Cohen-Kaplan *et al*.,(2012).

Table- 1 : HNSCC relevant cytokines and their proposed cellular functions

Cytokines	Sites of action
Basic fibroblast growth factor (bFGF)	Angiogenesis, metastasis
Granulocyte macrophage-colony-stimulating factor(GM-CSF)	CD34 mobilisation, immune suppression
IL-1	Cytokine secretion, gelantine production
IL-4	Immune suppression
IL-6	Inflammation regulation, anti-apoptosis
IL-8	Angiogenesis
IL-10	Immune suppression
Hepatocyte growth factor (HGF)	Angiogenesis
Macrophage migration inhibitory factor (MIF)	Growth regulation
Platelet-derived growth factor (PDGF)	Angiogenesis
Prostaglandin E2 (PGE2)	Immune suppression
Transformig growth factor-b (TGF-b)	Immune suppression
Vascular endothelial growth factor (VEGF)	Angiogenesis, metastasis, chemoattraction

Upon Activation by proinflammatory cytokines, suchas IL-1 and TNF-a,IkB kinases (IKKs) and Casein kinase 2 (CK2) can phosphorylate IkBs, which leads to ubiquitination And degradation of the IkBs by the 26S proteasome. This releases the bound NF-kB1/RelA and allows for its processing and translocation to the nucleus, resulting in the activation of multiple target genes Brown *et al.*,(2008). Sen *et al.,* (2012) .Constitutive activation of NF-kB dysregulates genes that regulate cell proliferation (cyclin D1), apoptosis and resistance to chemotherapeutics and radiation (IL-6, IL-8, cIAP1, Bcl-xL, YAP1), angiogenesis, immune, And proinflammatory responses (IL-6,IL-8,VEGF,HGF), and metastasis (IL-8, HGF, MMP9 Duan *et al*.,(2007), Allen *et al.,(*2008). Nuclear staining of the phospho-activated form of p65 correlates with decreased survival Rabinowich *et al.,* (1992). Head and neck cancer cells acquire the ability to subvert the chemokine (chemo-attractant cytokines) system, such that these molecules and their receptors become important regulators of cell movement into and out of the tumour microenvironment and major players in cancer biology. Balkwill (2012)

Cytokine-driven therapy

Cytokine-driven immunotherapy is a nonspecific, passive approach aiming to augment or boost immune cells with antitumor activity. Several cytokines have been used in therapy of SCCHN, as follows.

Interleukin 2

Interleukin (IL)-2 has been considered a key growth and death factor for antigen-activated T lymphocytes. IL-2 is also essential to maintain self-tolerance. ptor-deficient mice exhibit lethal autoimmunity. The intrinsic death-sensitizing activity of IL-2 was thought to be a key mediator for

apoptosis of peripheral autoreactive T cells. Interleukin-2 (IL-2) directly activates lymphocytes and sustains their proliferation. It has also been reported to be able to directly inhibit growth of SCCHN Sacchi *et al.*, (1991), Rabinowich *et al.*, (1992) . Injections of IL-2 in SCCHN patients demonstrated increased numbers and activities of both T cells and NK cells infiltrating the tumor stroma Whiteside *et al.*, (1993). Several subsequent clinical trials have combined systemic IL-2 with interferon (IFN)-α or chemotherapy and achieved various response rates, but the individual contribution of IL-2 to these responses was difficult to determine Agarwala *et al.*,(1991), Vlock *et al.*, (1991).

IFN-γ

Interferon γ (IFN- γ) up-regulates expression of several critical cellular molecules, including TAAs, adhesion molecules, and MHC class I and II molecules. A direct cytotoxic and cell-differentiation effect of IFN-c on SCCHN cell lines was demonstrated Richtsmeier (1998) . IFN- γ infusions in subsequent phase I/II clinical studies demonstrated notable responses in advanced SCCHN and induced tumor cell differentiation in several instances Ikic *et al.*,(1981), Richtsmeier *et al.*,(1990),. Recombinant DNA technology makes possible to produce clinical-grade IFN- γ and now it will be possible to reevaluate the clinical potential of this cytokine for patients with SCCHN.

Interleukin-12

Endogenous IL-12 is important for host resistance to tumors; the antitumor activity of IL-12 has been extensively reported in mouse models of cancer, where it has been shown to inhibit tumorigenesis and induce regression of established tumours'. The major antitumor activities of IL-12 rely on its ability to promote Th1 adaptive immunity and CTL responses. IFN-γ produced by naive Th cells also contributes to theantitumor activity of IL-12. IFN-γ has both a direct toxic effect on cancer cells and antiangiogenic activity. Lin and Karin (2007). injection of recombinant IL-12 into the primary tumor cause redistribution of lymphocytes from the peripheral blood to the lymph nodes in the neck; a significant increase in natural killer cells and a lower percentage of T_H cells in the lymph nodes and the primary tumor; and a 128-fold increase in IFN-γ mRNA in the lymph nodes Egan *et al.*, (2007). The use of IL-12 in cancer therapy, however, is hindered by severe toxic side effects. Injection of IL-12-loaded microspheres is more effective than repeated bolus injections of soluble IL-12 in promoting tumor suppression. Biodegradable microspheres may provide a safer and simpler alternative to current cytokine immunotherapies designed to deliver cytokines into the tumor microenvironment in a sustained manner.

Interferon-α

Interferon-α has been added to other drugs in the treatment of HNSCC. The combination of IFN-α, cisplatin, and 5-fluorouracil was associated with an overall response rate of 55% in patients with advanced esophageal cancer, accompanied by considerable toxicity Bazarbashi *et al.*, (2002) . In a phase 2 study of interferon-α plus isotretinoin and vitamin E in patients with locally advanced HNSCC, the 5-year progression-free survival rate was 80% and the 5-year overall survival rate was 81.3% Seixas-Silva *et al.*,(2005). Combination treatment with low dose recombinant IL-2 and interferon alpha-2α has also produced significant clinical tumor regressions in 2 of 11 (18%) heavily pretreated patients with recurrent disease Urba *et al.*, (1993). Recently, IFN-α in combination with 13-cis-retinoic acid (cRA) and α-tocopherol was used in an adjuvant phase II chemoprevention trial to prevent recurrence and/or secondary tumors, and it was found to be generally well tolerated.

Onco VEX$^{GM\text{-}CSF}$

Onco VEX$^{GM\text{-}CSF}$ is a second-generation oncolytic herpes simplex virus that delivers GM-CSF. In a phase 1 trial, multiple doses of Onco VEX$^{GM\text{-}CSF}$ were safe and well tolerated in patients with a range of solid tumor types, GM-CSF was expressed, and there was evidence of antitumor activity Hu *et al.*,(2006).

Cytokine mixtures

A complex Cytokine mixture (IRX-2) is primary cell derived biologic contains multiple cytokines: IL-1, 2, -6, and -8, tumor necrosis factor-α,IFN-γ,G-CSF,and GM-CSF. It is sterile, endotoxin-free, and serum-free, and is produced from purified human mononuclear cells that are stimulated by phytohemagglutinin

(PHA) under GMP conditions. This cytokine mixture remains a promising approach to immunotherapy of SCCHN. Meneses *et al*., (1998), Verastegui *et al*., (1997).

REFERENCES

Agarwala S, Vlock D, Johnson JT et al (1991). Phase II trial of interferon-alpha in locally recurrent or metastatic head and neck cancer: results of ECOG trial P-Z386. *Proceedings of the American Society for Clinical Oncology* **10** 205.

Allen C, Saigal K, Nottingham L, Arun P, Chen Z, Van Waes C (2008). Bortezomib-induced apoptosis with limited clinical response is accompanied by inhibition of canonical but not alternative nuclear factor-{kappa} B subunits in head and neck cancer. *Clinical Cancer Research* **14** 4175-4185.

Arti Yadav, Bhavna Kumar, Jharna Datta, et al., (2011). IL-6 Promotes Head and Neck Tumor Metastasis by Inducing Epithelial Mesenchymal Transition via the JAK-STAT3-SNAIL Signaling Pathway. *Molecular Cancer Research* **9** 1658-1667.

Arunabha Sengupta (2012). Recent advances in head and neck cancer. *Apollo Medicine* 1-8.

Banibrata Sen, Shaohua Peng, Denise M. Woods, et al., (2012). Stat5a Mediated Socs2 Expression Regulates Jak2 And Stat3 Activity Following C Src Inhibition In Head And Neck Squamous Carcinoma *Clinical Cancer Research* **18** 127-139.

Borish LC and Steinke JW (2003). Cytokines and chemokines. *Journal Allergy Clinical Immunology* **111.**

Brett C DiNatale, Jennifer C, Schroeder and Gary H Perdew (2011). Ah Receptor Antagonism Inhibits Constitutive and Cytokine Inducible IL6 Production in Head and Neck Tumor Cell Lines *Molecular Carcinogenesis* **50** 173–183.

Brown M, Cohen J, Arun P, Chen Z, Van Waes C (2008). NF kappa B in carcinoma therapy and prevention. *Expert Opinion on Therapy Targets* **12** 1109-1122.

Chen Z, Malhotra PS, Thomas GR et al., (1999). Expression of proinflammatory and proangiogenic cytokines in patients with head and neck cancer. *Clinical Cancer Research* **5** 1369–1379.

David D Walker, Travis D Reeves, Anna-Maria de Costa, Corinne Schuyler, M Rita I Young (2012). Immunological modulation by 1a 25-dihydroxyvitamin D3 in patients with squamous cell carcinoma of the head and neck. *Cytokine* **58** 448–454.

Douglas WG, Tracy E, Tan D et al., (2004). Development of head and neck squamous cell carcinoma is associated with altered cytokine responsiveness. *Molecular Cancer Research* **2** 585–593.

Duan J, Friedman J, Nottingham L, Chen Z, Ara G, Van Waes C (2007). Nuclear factor kappa B p65 small interfering RNA or proteasome inhibitor bortezomib sensitizes head and neck squamous cell carcinomas to classic histone deacetylase inhibitors and novel histone deacetylase inhibitorPXD101. *Molecular Cancer Therapy* **6** 37-50.

Duffy SA, Taylor JM, Terrell JE, Islam M, Li Y, Fowler KE, Wolf GT, Teknos TN (2008). Interleukin-6 predicts recurrence and survival among head and neck cancer patients. *Cancer* **113** 750-757.

Eisma RJ, Spiro JD, Kreutzer DL (1997). Vascular endothelial growth factor expression in head and neck squamous cell carcinoma. *American Journal Surgery* **174** 513–517.

Frances R Balkwill (2012). The chemokine system and cancer. *Journal of Pathology* **226** 148–157.

Franco Lumachi, Stefano MM Basso, Rocco Orlando (2010). Cytokines thyroid diseases and thyroid cancer. *Cytokine* **50** 229–233.

Ikic D, Padovan I, Brodarec I, Knezevic M, Soos E (1981) Application of human leucocyte interferon in patients with tumours of the head and neck. *Lancet* **1** 1025.

JA Seixas-Silva Jr, T Richards, FR Khuri *et al*., (2005). Phase 2 bioadjuvant study of interferon alfa2a isotretinoin and vitamin E in locally advanced squamous cell carcinoma of the head and neck: long-term follow-up. *Archives of Otolaryngology* **131** 304–307.

JE Egan, KJ Quadrini, F Santiago-Schwarz, J W Hadden, HJ Brandwein and KL Signorelli, (2007). IRX2 a novel in vivo immunotherapeutic induces maturation and activation of human dendritic cells in vitro. *Journal of Immunotherapy* **30** 624–633.

JC Hu, RS Coffin, CJ Davis, et al (2006). A phase I study of OncoVEXGMCSF a second generation oncolytic herpes simplex virus expressing granulocyte macrophage colony stimulating factor. *Clinical Cancer Research* **12** 6737–6747.

Lee TL, Yeh J, Friedman J, Yan B, Yang X, Yeh NT, Van Waes C, Chen Z (2008) . A signal network involving coactivated NF-kappaB and STAT3 and altered p53 modulates BAX/BCL-XL expression and promotes cell survival of head and neck squamous cell carcinomas. *International Journal of Cancer* **122** 1987-1998.

Leeman RJ, Lui VW, Grandis JR (2006). STAT3 as a therapeutic target in head and neck cancer. *Expert Opinion on Biological Therapy* **6** 231-241.

Medenica R, Slack N (1985). Clinical results of leukocyte interferon-induced tumor regression in resistant human metastatic cancer resistant to chemotherapy and/or radiotherapypulse therapy schedule. *Cancer Drug Delivery* **2** 53.

Meneses A, Verastegui E, Barrera JL, Zinser J, de la Garza J, Hadden JW (1998) Histologic findings in patients with head and neck squamous cell carcinoma receiving perilymphatic natural cytokine mixture (IRX-2) prior to surgery. *Archives of Pathology and Laboratory Medicine* **122** 447.

Naoki Umemura, Jianzhong Zhu, Yvonne K. Mburu et al., (2012). Defective NF-κB Signaling in Metastatic Head and Neck Cancer Cells Leads to Enhanced Apoptosis by Double-Stranded RNA. *Cancer Research* **72** 45-55.

Noda Y (2007). Interleukin-6 directly influences proliferation and invasion potential of head and neck cancer cells. *European Archives of Otorhinolaryngol* **264** 815-821.

Panomwat Amornphimoltham, Vyomesh Patel, Alfredo Molinolo and J. Silvio Gutkind (2011). Head and Neck Cancer and the PI3K/Akt/mTOR Signaling Network Novel Molecular Targeted Therapies *Signaling Pathways In Squamous Cancer* 407-429.

Rabinowich H, Vitolo D, Altarac S, Herberman RB, Whiteside TL (1992). Role of cytokines in the adoptive immunotherapy of an experimental model of human head and neck cancer by human IL-2-activated natural killer cells. *Journal in Immunology* **149** 340.

Richtsmeier WJ (1988). Interferon gamma induced oncolysis: an effect on head and neck squamous carcinoma. *Archives of Otolaryngoly Head Neck Surg* **(114)** 432.

Richtsmeier WJ, Koch WM, McGuire WP, Poole ME, Chang EH (1990) Phase I-II study of advanced head and neck squamous cell carcinoma patients treated with recombinant human interferon gamma. *Arch Otolaryngol Head Neck Surgery* **116** 1271.

S Bazarbashi, M Rahal, MA Raja et al., (2002). A pilot trial of combination cisplatin 5 fluorouracil and interferon alpha in the treatment of advanced esophageal carcinoma. *Chemotherapy* **48** 211–216.

SG Urba, AA Forastiere, GT Wolf and PC Amrein (1993). Intensive recombinant interleukin 2 and alpha interferon in patients with advanced head and neck squamous carcinoma. *Cancer* **71** 2326–2331 S460–S475.

Sacchi M, Klapan I, Johnson JT, Whiteside TL (1991). Anti proliferative effects of cytokines on squamous cell carcinoma. *Arch Otolaryngoly Head Neck Surgery* **117** 321.

Sutima Luangdilok , Carol Box, Kevin Harrington, Peter Rhy Evans, Suzanne Eccles (2011). MAPK and PI3K signalling differentially regulate angiogenic and lymphangiogenic cytokine secretion in squamous cell carcinoma of the head and neck. *European Journal of Cancer* **47** 520-529.

Van Waes C (2007). Nuclear factor kappa B in development prevention and therapy of cancer. *Clinical Cancer Research* **13** 1076-1082.

Verastegui E, Barrera JL ,Zinser J, DelRio R, Meneses A, De La Garza J, Hadden JW (1997). A natural cytokine mixture (IRX-2) and interference with immune suppression induce immune mobilization and regression of head and neckcancer. *International Journal of Immunology pharmacology* **19** 619.

Victoria Cohen-Kaplan, Jenny Jrbashyan, Yoav Yanir, Inna Naroditsky, Ofer Ben-Izhak, Neta Ilan, Ilana Doweck and Israel Vlodavsky (2012). Heparanase Induces Signal Transducer and Activator of Transcription (Stat) Protein Phosphorylation. *The Journal of Biological Chemistry* **287** 6668 –6678.

Vlock DR, Johnson J, Myers E, Day R, Gooding WE, Whiteside T, Pelch K, Sigler B, Wagner R, Colao D (1991). Preliminary trial of non-recombinant interferon-alpha in recurrent squamous cell carcinoma of the head and neck. *Head Neck* **13** 15.

Wan-Wan Lin and Michael Karin (2007). A cytokine-mediated link between innate immunity inflammation and cancer . *Journal of Clinical Investigation* **117** 1175–1183.

Whiteside TL, Letessier E, Hirabayashi H, Vitolo D, Bryant J, Barnes L, Snyderman C, Johnson JT, Myers E, Herberman RB et al., (1993). Evidence for local and systemic activation of immune cells by peritumoral injections of interleukin 2 in patients with advanced squamous cell carcinoma of the head and neck. *Cancer Research* **53** 5654.

Woods KV, El Naggar A, Clayman GL, Grimm EA (1998). Variable expression of cytokines in human head and neck squamous cell carcinoma cell lines and consistent expression in surgical specimens. *Cancer Research* **58** 3132–3141.

Worden B, Yang XP, Lee TL, Bagain L, Yeh NT, Cohen JG, Van Waes C, Chen Z (2005). Hepatocyte growth factor/scatter factor differentially regulates expression of proangiogenic factors through Erg 1 in head and neck squamous cell carcinoma. *Cancer Research* **65** 7071-7080.