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Role of Inflammation in Growth, Invasion and Metastasis of Oral Squamous Cell Carcinoma- A Molecular Insight

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ABSTRACT

Inflammatory conditions in selected organs increase the risk of cancer. Recent studies are unraveling molecular pathways linking inflammation and cancer. In the tumor microenvironment, smoldering inflammation contributes to proliferation and survival of malignant cells, angiogenesis, metastasis, subversion of adaptive immunity, reduced response to hormones and chemotherapeutic agents. In this review we have discussed role of various inflammatory molecules playing role in carcinogenesis and mainly reviewed the role of inflammation and inflammatory mediators in the growth, invasion and metastasis of oral squamous cell carcinoma.

Keywords: cancer, inflammation, angiogenesis, metastasis.

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INTRODUCTION

Inflammation (Latin, *inflammō*, "I ignite, set alight") is part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells, or irritants. The classical signs of acute inflammation are pain, heat, redness, swelling, and loss of function. Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process [1]. Without it, one probably wouldn't survive beyond infancy. While it is not possible to live without it, too much inflammation can cause serious damage. Chronic, persistent inflammation is behind a host of health problems such as rheumatoid arthritis and psoriasis [2].

It is for that reason that inflammation is normally closely regulated by the body. After finding immune cells in tumor samples, Rudolf Virchow was the first to ask whether inflammation might also contribute to cancer [2]. With extensive research in this field now we know that inflammation's dark side is a powerful force in cancer development by orchestrating the microenvironment around tumors, contributing to proliferation, survival and migration. On the other hand, many cells of the immune system contribute to cancer immunology, suppressing cancer.

In this study we have reviewed the role of inflammation in development, invasion, and metastasis of Oral squamous cell carcinoma (OSCC).

Role of inflammation in development of cancer

Link between inflammation and cancers, rather than a recent concern, was noticed ~150 years ago. As early as 1863, Virchow indicated that cancers tended to occur at sites of chronic inflammation. Lately, it turned out that acute inflammation contributed to the regression of cancer. However, accumulated epidemiologic studies support that chronic inflammatory diseases are frequently associated with increased risk of cancers [3].

Chronic inflammation conditions, caused by genetic mutations, autoimmune diseases, and exposure to environmental factors can increase the risk of cancer. Epidemiological studies have attributed up to 25% of cancer deaths worldwide to chronic inflammation. Chronic inflammation associated with microbial infections (*Helicobacter pylori*), autoimmune diseases (inflammatory bowel disease), inflammatory conditions of unknown origin (prostatitis) and smoking are well documented to increase the risk of certain cancers [3].

The inflammatory state is necessary to maintain and promote cancer progression and accomplish the full malignant phenotype, such as tumor tissue remodeling, angiogenesis, metastasis and the suppression of the innate anticancer immune response. In these cancers, inflammation is elicited by genetic and/or epigenetic mutation that triggers cell transformation and maintains the autonomous proliferation of the transformed cells [3].

Tumor-infiltrating leukocytes as well as cytokine related signaling pathways are critical components in the development of the inflammatory tumor microenvironment. Understanding the roles of each type of cell and signaling pathway involved in cancer initiation and progression is critical to the discovery of biomarkers specifically targeting cancer inflammation [3, 4]. Cancer related inflammation can fall into one of two categories:

- a. Precancerous inflammation lesions and
- b. Inflammation that is present in almost all cancer tissues including those that have no precancerous inflammation lesions [5].

Inflammatory conditions in selected organs increase the risk of cancer. An inflammatory component is present also in the microenvironment of tumors that are not epidemiologically related to inflammation. Recent studies have begun to unravel molecular pathways linking inflammation and cancer. In the tumor microenvironment, smoldering inflammation contributes to proliferation and survival of malignant cells, angiogenesis, metastasis, subversion of adaptive immunity, reduced response to hormones and chemotherapeutic agents. Recent data suggest that an additional mechanism involved in cancer-related inflammation (CRI) is induction of genetic instability by inflammatory mediators, leading to accumulation of random genetic alterations in cancer cells. In a seminal contribution, Hanahan and Weinberg identified the six hallmarks of cancer. CRI represents the seventh hallmark [6].

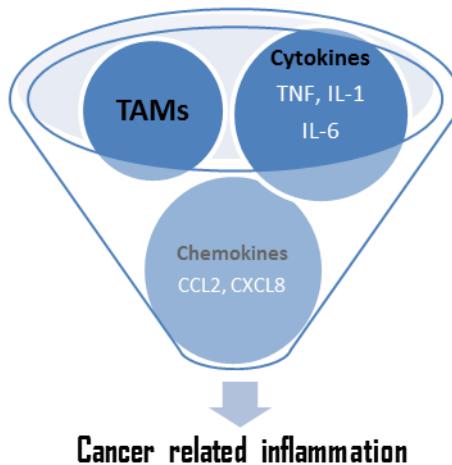


Figure 1: Diagrammatic representation for events that cause CRI.

Epidemiological studies have revealed that chronic inflammation predisposes to different forms of cancer. Usage of non-steroidal anti-inflammatory agents is associated with protection against various tumors, a finding that to a large extent mirrors that of inflammation as a risk factor for certain cancers. The 'inflammation cancer' connection is not restricted to increased risk for a subset of tumors. Key features of cancer-related inflammation (CRI) include the infiltration of white blood cells, prominently tumor-associated macrophages (TAMs); the presence of polypeptide messengers of inflammation (cytokines such as tumor necrosis factor

(TNF), interleukin (IL-1, IL-6), chemokines such as CCL2 and CXCL8) and the occurrence of tissue remodeling and angiogenesis [6].

Recent efforts have shed new light on molecular and cellular circuits linking inflammation and cancer. Two pathways have been schematically identified [6];

1. Intrinsicpathway: Genetic events causing neoplasia initiate the expression of inflammation-related programs that guide the construction of an inflammatory microenvironment (e.g. RET oncogene in papillary carcinoma of the thyroid). Oncogenes representative of different molecular classes and mode of action share the capacity to orchestrate proinflammatory circuits (e.g. angiogenetic switch; recruitment of myelomonocytic cells).
2. Extrinsicpathway: Inflammatory conditions facilitate cancer development. The triggers of chronic inflammation that increase cancer risk or progression include infections (e.g. Helicobacter pylori for gastric cancer and mucosal lymphoma; papilloma virus and hepatitis viruses for cervical and liver carcinoma, respectively), autoimmune diseases (e.g. inflammatory bowel disease for colon cancer) and inflammatory conditions of uncertain origin (e.g. prostatitis for prostate cancer).

Key orchestrators at the intersection of the intrinsic and extrinsic pathway include transcription factors and primary proinflammatory cytokines. Thus, CRI is a key component of tumors and may represent the seventh hallmark of cancer [6, 7]. Cancer cells use selectins, chemokines and their receptors for invasion, migration and metastasis.

Molecular intersection between receptors of steroid hormones, which have important effects on cellular development, and transcription factors that play key roles in inflammation, such as NF- κ B, may mediate some of the most critical effects of inflammatory stimuli on cancer cells [1].

This capacity of a mediator of inflammation to influence the effects of steroid hormones in cells, is very likely to affect carcinogenesis on the one hand; on the other hand, due to the modular nature of many steroid hormone receptors, this interaction may offer ways to interfere with cancer progression, through targeting of a specific protein domain in a specific cell type. Such an approach may limit side effects that are unrelated to the tumor of interest, and may help preserve vital homeostatic functions and developmental processes in the organism [1].

NF- κ B induces the expression of inflammatory cytokines, adhesion molecules, key enzymes in the prostaglandin synthase pathway (COX-2), nitric oxide (NO) synthase and angiogenic factors. In addition, by inducing antiapoptotic genes (e.g. Bcl2), it promotes survival in tumor cells and in epithelial cells targeted by carcinogens [6].

In a study by F. Collotta, IL-6 promotes liver inflammation, injury, compensatory cell proliferation and carcinogenesis. In females, estrogen steroid hormones inhibit IL-6 production and so protect female mice from cancer.

Among proinflammatory cytokines, TNF plays a major role. Originally identified as a cytokine inducing hemorrhagic necrosis of tumors, TNF soon turned out to have also protumoral functions. The finding that TNF-deficient mice are protected from skin carcinogenesis offered genetic evidence linking TNF-mediated inflammation and cancer. Tumor promotion by this cytokine can involve different pathways: TNF enhances tumor growth and invasion, leukocyte recruitment, angiogenesis and facilitate epithelial to mesenchymal transition [6].

Together with TNF and IL-6, IL-8, also IL-1 has long been known to augment the capacity of cancer cells to metastasize, by affecting multiple steps of the CRI cascade [6].

TAM (tissue associated macrophages) assists tumor cell malignant behavior in many ways by releasing cytokines, growth factors and matrix-degrading enzymes and a host of angiogenic factors (e.g. vascular endothelial growth factor (VEGF), platelet-derived growth factor, fibroblast growth factor and CXCL8) [6].

Monocytes express VEGF receptors and VEGF is a known chemo attractant of myeloid cells in tumors. VEGFR+ hematopoietic cells home to tumor-specific premetastatic sites that favor secondary localization of cancer [6].

Tumor progression is largely mediated by the host inability to mount a protective antitumor immune response [6].

Metabolic changes in the tumor milieu, in addition to provide growth and survival advantages for cancer cells, may also influence infiltrating leukocytes. It was found that lactic acid secreted by tumor cells promotes the IL-23/IL-17 axis in TAM. Thus, lactic acid is a proinflammatory stimulus inducing the IL-23/IL-17 pathway to the expenses of the immunoprotective IL-12-inducible Th1 pathway [6].

In the extrinsic pathway, it remains uncertain whether chronic inflammation per sé is sufficient for carcinogenesis. Reactive oxygen and nitrogen intermediates are obvious inflammation-generated candidate mediators for DNA damage and evidence obtained in vitro and in vivo is consistent with this view [6].

Growth factors and chemokines produced by inflammatory cells in tumor microenvironment induce overexpression of structurally normal c-Myc in cancer cells. c-Myc alters the expression of hundreds of target genes related to cell growth, apoptosis and invasion. However, c-Myc also accelerates the intrinsic mutation rate in cancer cells [6].

Is inflammation associated with genetic instability in non-cancer conditions?

The concept that an inflammatory microenvironment contributes to genome destabilization in cancer is in keeping with findings of microsatellite instability and Chromosomal instability also in non-cancer-related inflammatory conditions. The mutation rate in the inflamed microenvironment is higher than in normal tissues, with a mutation frequency of 4×10^{-8} and $<1 \times 10^{-8}$ per base pair, respectively [6].

DISCUSSION

Inflammation in oral squamous cell carcinoma:

Chronic inflammation leading to cancer is an example of dysregulation of an essential process becoming hazardous. Innate immunity, which is the first line of the host defense against a variety of insults, protects cells through the release of inflammatory mediators, such as cytokines, chemokines, matrix-remodeling proteases, and reactive oxygen species [8,9]. However, malfunctioning immune components could lead to chronic inflammation, generating a microenvironment that may initiate and promote carcinogenesis [10].

Oral squamous cell carcinoma (OSCC) is the sixth most common cancer and an important public health concern worldwide, with ~405,000 new cases and 211,000 deaths reported annually. Patients diagnosed with oral cancer have a particularly low 5-year survival rate due to the compounding factors of late detection and lack of truly effective therapies according to the American Cancer Society and the online Facts page of The Oral Cancer Foundation. Therefore, development of early detection techniques and subsequent innovative therapies are greatly needed. Besides high mortality, OSCC is also often associated with eating difficulties, speech impairment, and general psychological distress. Tobacco and alcohol consumption, betel quid chewing, and viral infections are some of the known risk factors for OSCC. In addition, oral infections leading to periodontal diseases are also associated with OSCC. About 20% of oral leukoplakia undergo malignant transformation and develop into OSCC [11].

Chronic inflammation is associated with the development of a variety of epithelial cancers such as colon and pancreatic cancers, but whether it plays a significant role in development of oral cancers is unclear. It is believed that the chronic inflammatory environment causes genomic alterations that eventually lead to tumor development. Essential components in this association are the cytokines produced by tumor cells themselves as well as by the innate immune cells activated during the inflammatory process [11].

Thais Helena Gasparoto et al used a multistage model of SCC to examine the involvement of elastase (ELA), myeloperoxidase (MPO), nitric oxide (NO), cytokines (IL-6, IL-10, IL-13, IL-17, TGF- β and TNF- α), and neutrophils and macrophages in tumour development. They showed that ELA and MPO activity and NO, IL-10, IL-17, TNF- α and TGF- β levels were increased in the precancerous microenvironment [12].

Additionally, neutrophil infiltrate was positively correlated with MPO and NO levels in the lesions [12].

Increase of inflammatory mediators such as NO, active MPO and ELA, which are up-regulated in response to chronic inflammation, can increase mutation rates by inducing DNA damage and genomic instability, in addition to enhancing the proliferation of mutated cells. These events are associated with tumor initiation and progression, suggesting that inflammatory mediators may play an important role in initiation and promotion phase of SCC development. These findings represent a significant step towards carcinogenesis [12].

Our results suggest that activated neutrophils and macrophages are involved in inflammatory mediator production in tumor microenvironment. These cells may drive some immunity-related skin tissue damage and support cancer establishment [12].

Del Prete A et al in their article have discussed that key orchestrators at the intersection of the intrinsic and extrinsic pathways include transcription factors (e.g. Nuclear Factor kappa-B, NF- κ B) that modulate the inflammatory response through soluble mediators (cytokines, chemokines) and cellular components (e.g. tumor-associated macrophages), promoting tumorigenesis. NF- κ B aids in the proliferation and survival of malignant cells, promotes angiogenesis and metastasis, subverts adaptive immunity, and alters responses to hormones and chemotherapeutic agents [13].

Rao S K et al in their study published microarray analysis of gene expression in OSCC cell lines with high basal NF- κ B activity and OSCC patient samples identified dysregulation of many genes involved in inflammation, wound healing, angiogenesis, and growth regulation. In particular IL-8, CCL5, STAT1, and VEGF gene expression was up-regulated in OSCC. Moreover, IL-8 protein levels were significantly higher in OSCC cell lines as compared with normal human oral keratinocytes [11].

According to Yan M et al the oral squamous cell carcinoma cell lines with highly metastatic potential cells had high level of constitutive NF- κ B activity and were more sensitive to TNF-alpha. They concluded that the inflammatory factors such as TNF-alpha could promote oral squamous cell carcinoma cell metastasis [14].

According to Wanlu Lu, Libing Lu et al, the inflammatory cells and molecules within the tumor microenvironment have decisive dual roles in antitumor immunity and immune evasion. In their study, phytohemagglutinin (PHA) was used to stimulate peripheral blood mononuclear cells (PBMCs) to simulate the tumor inflammatory microenvironment. The effect of immune cells and inflammatory cytokines on the surface expression of programmed cell death-1 ligand 1 (PD-L1) and tumor immune evasion was investigated using flow cytometry (FCM) and an in vivo xenotransplantation model. Based on the data, PHA-activated, but not resting, immune cells were able to promote the surface expression of PD-L1 in Tca8113 oral squamous carcinoma cells via the secretion of inflammatory cytokines, but not by cell-cell contact. These results indicate a new mechanism for the promotion of tumor immune evasion by the tumor inflammatory microenvironment [15].

Peritumoral inflammatory infiltrate in oral squamous cells carcinoma:

Meneses et al., (1998) demonstrated, in oral squamous cell carcinoma samples, a possible association between tumor size, area of invasion, angiogenesis, and the phenotypical characterization of the peritumoral inflammatory infiltrate predominantly comprised by T lymphocytes and B lymphocytes [16].

Coussens et al., (1999) also found in carcinomas of oral mucosa an association between the predominance of mast cells in the peritumoral infiltrate and a greater development of the stromal angiogenesis, which would provide adequate blood supply for neoplastic nutrition, and consequently, a poorer prognosis [17].

Fabricio LD, Vieira et al from their study results suggested that the cellular immune response is the main defense mechanism in squamous cell carcinoma of oral mucosa, expressed by the large number of T lymphocytes and macrophages, and that the greatest intensity of local response may be associated with the best prognosis [18].

CONCLUSION

Despite the exciting advances in the field of cancer inflammation research, many questions remain. The cross talk between the different signaling pathways involved in cancer-related inflammation is an area that remains unclear. This is not surprising based on the high heterogeneity of genetic and epigenetic alterations present in different cancers, differences in host genetic background as well as tissue specific inflammatory responses. Identification of the molecular targets in OSCC and subsequent innovative therapies are greatly needed. Emerging evidence suggests that persistent inflammation promotes genetic instability. Thus, cancer-related inflammation represents a target for innovative diagnostic and therapeutic strategies.

The study results of various authors regarding the role of peritumoral inflammatory infiltrate with the tumor differentiation and prognosis are not in concordance with other. More studies are needed in this field on larger samples.

Defining the roles of inflammatory mediators and the underlying signaling pathways will be critical to increasing the understanding of cancer initiation and progression. This will aid in the discovery of biomarkers for disease stratification, molecular diagnosis & prognosis, therapy selection and drug development.

Table 1: Various molecular factors and their role in tumor development.

FAMILY FACTORS	OF	MOLECULAR FACTORS	FUNCTION	ROLE IN TUMOR DEVELOPMENT
Transcription factors		Nuclear factor-kappa B ⁶	Induces expression of inflammatory cytokines, adhesion molecules, Key enzymes in the prostaglandin synthase pathway (COX-2), nitric oxide (NO) synthase and angiogenic factors ⁶	Tumor initiation and progression ⁶
		Signal transducer activator of transcription-3 (Stat3) ⁶	Induces convergence for numerous Oncogenic signaling pathways ⁶	Tumor immune evasion Cell proliferation Survival, Regulating the expression of c-Myc, Mcl-1, Cyclin D and Bcl-2 ⁶
		STAT1		OSCC development and/or progression ¹¹
Primary inflammatory Cytokines		IL-1 β , IL-6 ^{6,12} IL-8	Growth-promoting and anti-apoptotic activity	Promote inflammation-propelled neoplasia
		IL-10, IL-13, IL-17, TGF- β ¹²	Pro-tumor local tissue alterations	Carcinogenesis and metastasis through degradation of the extracellular matrix ¹²
		TNF- α ^{6,12}		TNF enhances tumor growth and invasion, leukocyte recruitment, angiogenesis and facilitate epithelial to mesenchymal transition ^{6,12}
Proinflammatory Chemokines		KC CXC, JE CCL2 and CCL3 ⁶	Maintenance of NF- κ B activation in tumors ⁶	Promote inflammation-propelled neoplasia ⁶
		CCL5 ¹¹		OSCC development and/or progression ¹¹
Miscellaneous factors		elastase (ELA) ¹² , myeloperoxidase (MPO) ¹² , nitric oxide (NO) ¹²	Pro-tumour local tissue alterations	Involved with carcinogenesis and metastasis through degradation of the extracellular matrix, facilitating cancer invasion ¹²
		VEGF gene ¹¹		Seen to Increase Cell Viability In OSCC Cell Lines ¹¹

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