Genomic Instability, Inflammation, and Cancer
Genomic Instability, Inflammation, and Cancer

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Editorial

Genomic Instability, Inflammation, and Cancer

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Accumulating evidence supports that the inflammatory milieu plays a key role in the initiation and progression of epithelial cancer, the most characteristic example being that of ulcerative colitis which is associated with increased risk of colonic adenocarcinoma. Other less frequent and studied inflammatory lesions include asbestos-induced cellular damages, oral lichen planus (premalignant lesion), and cardiac myxoma. The mechanistic basis of this association is just beginning to be clarified. In the center of this picture lies the cytokine network which seems to link genomic instability, an evolving hallmark of cancer, with cancer development. In this special issue which deals with genomic instability, inflammation, and cancer, several research groups present evidence supporting this connection.

H. Matsuzaki et al. have demonstrated a relationship between chronic inflammation and carcinogenesis through asbestos-induced cellular and molecular alteration of immunocompetent cells, resulting in a decline in tumor immunity. Induction of chronic inflammation in the areas of the lung, regional lymph nodes and the pleural cavity has been shown to be due to the production of reactive oxygen/nitrogen species. Cellular and molecular features of immunocompetent cells may be altered by asbestos fibers which, combined with the surrounding inflammation, eventually lead to decreased tumor immunity.

M. Murata et al. have investigated the role of nitrative and oxidative DNA damage in inflammation-related carcinogenesis. In their work they revealed that infectious and noninfectious agents induce iNOS-dependent formation of 8-nitroguanine and 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in cancer tissues and precancerous regions. Their results suggest that DNA base damage may lead to double-stranded breaks. They also demonstrated IL-6-modulated iNOS expression via STAT3 and EGFR in Epstein-Barr virus-associated nasopharyngeal carcinoma and found promoter hypermethylation in several tumor suppressor genes, while proposing 8-nitroguanine as potentially useful biomarkers for predicting the risk of inflammation-related cancers.

I. S. Pateras et al. have investigated the presence of herpes simplex virus (HSV) DNA in a cohort of cardiac myxomas assessing the possibility that HSV infection might be involved in the development of these lesions with potential therapeutic applications.

E. Georgakopoulou et al. have examined whether oral lichen planus exhibits malignant potential, with relation to the development of oral squamous cell carcinoma, and may therefore represent a model of preneoplastic inflammation.

I. Aivaliotis et al. have reviewed the effects of cytokines as crucial components of inflammation, participating in the interaction between the cells of the tumor microenvironment, and have focused on their potential role in the development of genomic instability.

This special issue of the Journal of Biomedicine and Biotechnology is devoted to the presentation of such inflammatory conditions with predisposition to genomic instability and cancer development. I hope that it will help stimulate research that is aimed at understanding and therapeutic targeting of these lesions.

Vassilis G. Gorgoulis
Review Article

How Do Cytokines Trigger Genomic Instability?

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Inflammation is a double-edged sword presenting a dual effect on cancer development, from one hand promoting tumor initiation and progression and from the other hand protecting against cancer through immunosurveillance mechanisms. Cytokines are crucial components of inflammation, participating in the interaction between the cells of tumor microenvironment. A comprehensive study of the role of cytokines in the context of the inflammation-tumorigenesis interplay helps us to shed light in the pathogenesis of cancer. In this paper we focus on the role of cytokines in the development of genomic instability, an evolving hallmark of cancer.

1. Introduction

Contemporary approaches in cancer research have been influenced by the accumulating data unveiling the importance of inflammatory components in the tumor microenvironment. It is becoming more clearly evident that inflammation demonstrates a dualism effect on cancer development in close resemblance to a ying-yang pattern. Inflammation may exhibit either a pro- or an antitumorigenic effect. Cytokines possess a central role in the inflammatory component implicated in the interplay between the host’s stromal cells and the tumor cells during tumorigenesis. In this paper we are shedding light on the molecular pathways linking cytokines with the induction of genomic instability, an evolving hallmark of cancer.

2. Interrelation of Inflammation and Carcinogenesis

Rudolf Virchow was the first to observe, back in the nineteenth, the presence of leukocytes inside tumors and this observation was the first indication of a possible linkage between inflammation and cancer. The last decade intensive research has focused on the molecular pathways involved in the above linkage and it is now well understood that chronic inflammation plays a significant role in the carcinogenesis process [1].

In 1909, Paul Ehrlich proposed the immunosurveillance theory, later established by Thomas and Burnet, which supports the tumor suppressive role of the immune system [2–4]. Dunn and his colleagues suggested in 2004 that a new theory should be adopted to describe the relationship between the immune response and tumorigenesis, called immunoeediting [5]. According to this theory, three distinct stages exist describing the interrelation between immunity and carcinogenesis. The first stage, termed elimination, represents the period in which the immune system, through successful immunosurveillance, destroys precancerous and cancerous cells. In equilibrium, the second stage, cancer cells have begun to develop abilities to avoid immunosurveillance mechanisms but the balance between “immune patrol” and tumorigenesis is still preserved. In the third stage, named
escape, the cancer cells manage to evade the surveillance system of the organism, resulting in aberrant cell proliferation and tumor development. Interestingly, it seems that the immune response to the tumor causes an "immunosculpting" effect on cancer cells that enables them to resist immunological recognition or to exert enhanced defense mechanisms against immunosurveillance [5].

Recent advances in cancer biology research have demonstrated that a chronic indolent inflammation environment harbors potential tumor promoting mechanisms [1]. According to Hanahan and Weinberg, one of the emerging hallmarks of cancer is the ability to escape immunosurveillance and an enabling characteristic for the acquisition of these capabilities is the inflammation propagated by the tumor [6]. Compelling evidence of the last decade supports the notion that the inflammatory microenvironment is important for the survival of tumors [1]. It seems that inflammatory cells of the innate immunity usually display a tumor promoting role whereas cells of adaptive immunity appear to have a tumor suppressive effect [1, 7].

Inadequate pathogen eradication or continuous exposure to chemical carcinogens preserve a chronic inflammation environment that may enhance tumorigenesis [8]. There is evidence supporting that several unresolved inflammatory reactions following persistent pathogen infection promote human malignancies [9]. Pathogens contain specific patterns, known as pathogen-associated molecular patterns (PAMPs), which are recognized by host receptors, named pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), nucleotide-binding oligomerization domain-like receptors (NOD-like) receptors, C-type lectin receptors (CLR.s), and triggering receptors expressed on myeloid cells (TREMs) [10, 11]. The binding between PAMPs and PRRs leads to inflammation-related cell activation and triggers host immune defense mechanisms against foreign pathogens [10]. In relation to the previous part, it is well established that chronic viral hepatitis B and C is strongly associated with the development of hepatocellular carcinoma. In this case, excessive host reaction towards the viral infection is believed to play a significant role for the inflammation-mediated liver carcinoma. On the other hand and not mutually exclusive there are several viral infections in which the virus itself through its oncogenic potential is mainly responsible for the cancer development [12]. Human Papilloma Virus (HPV) infection is associated with cervical cancers and Epstein-Barr infection bears significant association with Burkitt lymphoma and nasopharyngeal carcinoma. Particular types of HPV produce the E6 and E7 oncoproteins which interfere with the p53 and Retinoblastoma protein (pRb) pathways, respectively. Epstein-Barr virus (EBV) latent membrane protein 1 (LMP1) is critical for EBV-induced cellular transformation through the activation of NF-kB (analyzed hereinafter), a transcription factor promoting cell survival [13].

In the context of chemically induced carcinogenesis, a typical example involves cigarette smoking, in which the tumorigenic activity is partially attributed to its ability to induce chronic inflammation [14]. Also asbestos or silica exposure may cause inflammation of the lung and subsequent bronchial carcinoma [15]. The mechanism of the induced inflammation by the above inhaled particles occurs by means of prointerleukin-1β (IL-1β) processing by the inflammasome [1, 16]. The inflammasome is a protein complex which includes two caspase-1 molecules, is related to cryopyrin protein, and leads to IL-1β cross-activation and maturation [17].

3. Cellular Context and Cytokine Signaling in the Tumor Microenvironment

The cellular context of the tumor’s microenvironment includes cancer cells and surrounding stromal cells (involving fibroblasts, endothelial cells, pericytes, and mesenchymal cells) along with the infiltrating cells of the innate and adaptive immunity [1]. Innate immune cells include macrophages, myeloid-derived suppressor cells (MDSCs), neutrophils, and mast, dendritic, and natural killer (NK) cells, while adaptive immune cells consist of T and B lymphocytes. The only immune cells with no known tumor promoting role to date are NK cells [1]. MDSCs share common characteristics with macrophages, neutrophils, and dendritic cells and they help in tumor angiogenesis as well as suppression of antitumor immune responses [18, 19]. Tumor-associated macrophages (TAMs) and T cells are among the most frequently observed cells within the tumor microenvironment. TAMs are a heterogeneous cell population which evidently exerts a very significant role in tumor cell survival, growth, and progression and can be considered obligate partners for tumor cell migration, invasion, and metastasis [20, 21].

The interaction between the epithelial and stromal cells comprising the inflammatory microenvironment is mediated by a class of molecules named as cytokines. Cytokines are cell-signaling protein molecules with effects on intercellular communication; they include interleukins, lymphokines, and chemokines. Interleukins were initially observed to be expressed by leukocytes and present immunoregulatory action. Lymphokines are produced by lymphocytes and include IL-2, IL-3, IL-4, IL-5, IL-6, GM-CSF (Granulocyte-macrophage colony-stimulating factor), and IFN-γ (Interferon-gamma). Chemokines are chemoattractant cytokines and are named this way due to their ability to control leukocyte migration to inflammation sites through chemotaxis [22, 23]. The nomenclature of chemokines is based on the number and location of the N-terminal cysteine residues, divided in four different groups: CXC, CC, CX3C, and C [23].

In accordance with increased expression of pro- or anti-inflammatory cytokines and to tumor progression, TAMs may be classified into M1 and M2 types. M1 macrophages are mostly found in early stage tumor development and may release proinflammatory cytokines (TNF-a, IL-1, IL-6, IL-12, IL-23) and chemokines CXCL19 and CXCL10. Their physiological role involves Th1/Th17 cellular responses and NK cell development and differentiation. M2 macrophages may be mostly found in more established tumors, show increased expression of IL-10 and transforming growth...
factor β (TGF-β) as well as chemokines CCL17, CCL22, and CCL24, and are considered to promote tumor angiogenesis. This subtype of TAMs encourages Th2 and regulatory T cells (Tregs) recruitment [1, 7]. According to their effector functions, T cells can be subdivided in CD8+ cytotoxic T cells (CTLs) and CD4+ helper T (Th) cells, including Th1, Th2, and T regulatory (Treg) cells [1]. T cells may exhibit either antitumor immune responses or promote tumor growth [1, 24].

The mechanisms of inflammation-mediated tumor promotion involve secretion of specific cytokines by both inflammatory and tumor cells as well as activation of transcription factors, mainly (Nuclear Factor-κB) NF-κB, (Signal Transducers and Activators of Transcription) STAT3, and (Activator Protein-1) AP1. NF-κB and STAT3 expression can be detected in most cancers and these transcription factors activate genes responsible for cell survival, proliferation, angiogenesis, invasiveness, and production of cytokines [1, 10, 25, 26]. NF-κB belongs to a family of transcription factors that regulate the secretion of many inflammatory cytokines, specific adhesion molecules, and the prostaglandin biosynthetic pathway. It also regulates the expression of antiapoptotic proteins and angiogenic factors in a tissue-specific manner [15, 27]. There are three distinct activation pathways of NF-κB, the classical, the alternative, and the atypical pathways, and all seem to support tumorigenesis [18, 28, 29]. The classical pathway is triggered by pathogen infection, T-cell receptor (TCR) engagement, and proinflammatory cytokines, such as IL-1 and TNF-α [28, 29]. The NF-κB alternative pathway is triggered by cytokines belonging to the TNF family and involves the IKKα homodimer and the p52/RelB transcription factors [18, 28]. Finally, the atypical NF-κB pathway is IKK independent and is triggered by several stimuli such as hypoxia and hydrogen peroxide attack of the cells [29]. STAT3 activation induces the expression of Bcl2 and Bcl-XL antiapoptotic genes, Cyclin D1 or c-Myc proliferation genes, and VEGF (vascular endothelial growth factor) angiogenesis promoting gene [18, 27]. AP1 is a dimeric transcription factor composed of members of the Jun, Fos, activating transcription factor (ATF) and musculoaponeurotic fibrosarcoma (Maf) protein families. Several growth factors and cytokines induce MAPK signalling pathway which in turn activates AP1 [30].

Cytokine IFN-γ most frequently produced by cytotoxic CD8+ and CD4+ Th1 T cells has been recognized as a dominant tumor-inhibitory force (Table 1). On the other side, the cytokines interleukin-6 (IL-6), TNF-α, IL-1β, and IL-23 are mostly considered as tumor promoting [7]. Secretion of the latter cytokines is mainly induced by tumor associated macrophages and myeloid-derived suppressive cells [7]. One of the previously mentioned cytokines, macrophage-derived IL-1, was indicated to promote both inflammation and angiogenesis under a hypoxic environment which in turn ascerts the important role of IL-1 in inflammation-mediated tumorigenesis [31]. Additionally, IL-1β has recently been shown to induce a subset of MDSC in the tumor microenvironment capable of suppressing the development and function of NK cells [32]. Proinflammatory cytokines include IL-2, IL-6, IL-11, IL-15, IL-17, IL-23, TNF-α, and chemokine IL-8 and anti-inflammatory cytokines include IL-4, IL-10, IL-13, transforming growth factor β (TGF-β) and interferon (IFN)-α [22, 33]. Proinflammatory cytokines, such as IL-1 and TNF-α which in turn stimulate IL-8, may also stimulate chemokines [22, 34].

An interesting example of cytokine-mediated carcinogenesis is human malignant mesothelioma which may be provoked by chronic exposure to asbestos. TNF-α displays a tumor promoting role in this type of cancer by helping the survival of the mesothelial cells which, in turn, have been damaged by asbestos exposure and may develop mutagenic phenotypes rendering them susceptible to carcinogenesis [10, 35]. TNF-α favors the survival of tumor cells by inducing the expression of antiapoptotic genes encoded in an NF-κB-dependent manner [10]. As a result, TNF-α— which may be released by host as well as cancer cells—significantly influences the initiation and progression stages of all cancer types [10]. Similarly, IL-6 exerts a tumor promoting role by enhancing cell cycle progression and suppressing apoptosis. Its signaling transduction pathway is induced by STAT3 transcription factor [10, 21]. IL-17 triggers the secretion of TNF-α, IL-6, and IL-1β proinflammatory cytokines and is produced by IL-23-dependent STAT3 activation [10, 36]. IL-23 has similar functions to IL-17, induces IFN-γ, and IL-12 production by activated T cells, and leads to enhanced proliferation of memory T cells [10, 37]. IL-10 activates STAT3 transcription factor but has an opposite effect in carcinogenesis to IL-6 [10]. Additionally it inhibits NF-κB activation and in this manner also inhibits production of TNF-α, IL-6, and IL-12 [10, 38]. IL-10 probably exerts its tumor suppressive role by inhibiting the production of the above mentioned proinflammatory cytokines [10]. IL-10 may also exert protumorigenic activity through STAT3 activation by upregulating Bcl2 and Bcl-xL antiapoptotic genes, therefore conﬁrming its dual role in the process of carcinogenesis [10, 39]. Other cytokines have a known dual function in carcinogenesis (Table 1). Interestingly, TGF-β may exert a tumor suppressive role in the beginning of the process and a tumor promoting role at the late stages of carcinogenesis [40].

Lessons for the mechanisms implicated in inflammation-associated carcinomas have paradoxically been taken by the study of cellular senescence [41]. It has been shown that senescent cells are metabolically active and secrete several factors that may alter their own as well as the tumor microenvironment [42]. The phenomenon of such secretion by the senescence cells has been termed senescence-associated secretory phenotype (SASP) [43]. SASP acts in a cell-autonomous paracrine manner and has both a bright side, favoring senescence in normal or low-grade preneoplastic cells, as well as a dark side, facilitating evasion of senescence in high grade preneoplastic or cancerous cells [44]. The SASP involves a number of inflammatory cytokines, such as IL-6 and chemokine IL-8, which constitute two of its prominent components [43]. It has been demonstrated that the proinflammatory cytokines IL-6 and IL-8 are upregulated by persistent DNA Damage Response (DDR) activation which in turn boost the DDR signaling pathway forming a positive feedback loop [43]. Of note, IL-6 and IL-8 are known to play
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<th>Protumorigenic role</th>
<th>Antitumorigenic role</th>
<th>Unspecified yet role in tumorigenesis</th>
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<tr>
<td>IL-1 (α and β)</td>
<td>Tumor growth, invasion and metastasis, mainly through the action of IL-1β in promoting local inflammatory responses as well as angiogenesis.</td>
<td>Restraint of tumor growth through activation of innate and specific immune effector mechanisms mainly through the action of IL-1α.</td>
<td></td>
<td>[17]</td>
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<tr>
<td>IL-2</td>
<td>(i) Stimulates growth, differentiation, and survival of cytotoxic T cells (ii) Induces differentiation and proliferation of NK cells</td>
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<td>[53]</td>
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<tr>
<td>IL-3</td>
<td>Stimulation of activated B-cell and T-cell proliferation, and differentiation of CD4+ T-cells into Th2 cells</td>
<td>IgE and class II MHC expression on B cells</td>
<td></td>
<td>[54]</td>
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<tr>
<td>IL-4</td>
<td>(i) Decreases the production of Th1 cells, macrophages, and IFN-gamma (ii) Has been shown to drive dedifferentiation, mitogenesis and metastasis in rhabdomyosarcoma</td>
<td>Stimulation of activated B-cell and T-cell proliferation, and differentiation of CD4+ T-cells into Th2 cells</td>
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<td>[55]</td>
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<tr>
<td>IL-5</td>
<td>(i) Stimulates B cell growth (ii) Stimulates eosinophil growth and function</td>
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<td>[56]</td>
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<td>IL-6</td>
<td>(i) Promotion of tumor cell proliferation and inhibition of their apoptosis through activation of STAT-3. (ii) Facilitation of senescence evasion in high-grade preneoplastic or cancerous cells through mechanisms of SASP. (iii) Favours metastasis</td>
<td>(i) Mediator of the acute phase response (ii) Induction of senescence in normal or low grade preneoplastic cells.</td>
<td></td>
<td>[25, 44]</td>
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<tr>
<td>IL-7</td>
<td>Stimulation of proliferation of B cells, T cells, and NK cells</td>
<td>Stimulation of proliferation of B cells, T cells, and NK cells</td>
<td>Stimulation of proliferation of B cells, T cells, and NK cells</td>
<td>[57]</td>
</tr>
<tr>
<td>IL-8</td>
<td>Significant role in tumor growth, angiogenesis, epithelial to mesenchymal transition (EMT) and invasiveness</td>
<td>(i) Induction of chemotaxis in its target cells (neutrophils, granulocytes) (ii) Induction of senescence in normal or low-grade preneoplastic cells.</td>
<td></td>
<td>[43, 44]</td>
</tr>
<tr>
<td>IL-9</td>
<td>(i) Potential role in tumorigenesis due to antiapoptotic and growth factor activities (ii) Deregulated IL-9 response may lead to malignant transformation through Jak/STAT activation</td>
<td></td>
<td>Regulation of hematopoietic cells</td>
<td>[58]</td>
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<td>Cytokine</td>
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<td>Antitumorigenic role</td>
<td>Unspecified yet role in tumorigenesis</td>
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<td>IL-10</td>
<td>Potential tumor promoting activity through activation of STAT3 and consequent upregulation of BCL-2 or BCL-XL antiapoptotic genes.</td>
<td>(i) Enhances B-cell survival, proliferation, and antibody production. (ii) Inhibition of tumor development and progression through inhibition of NF-κB activation, TNF-α, IL-6, and IL-12. (iii) Suppression of angiogenesis through inhibition of the tumor stroma.</td>
<td>[10, 59]</td>
<td></td>
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<tr>
<td>IL-11</td>
<td>(i) Regulator of haematopoiesis (ii) Stimulation of megakaryocyte maturation</td>
<td>(i) Stimulates the growth and function of T cells (ii) Stimulates the production of IFN-γ, TNF-α (iii) Induces cell-mediated immune responses (iv) Exhibits antiangiogenic activity</td>
<td>[60]</td>
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<td>IL-12</td>
<td>(i) Stimulates growth, differentiation and survival of cytotoxic T cells (ii) Induces differentiation and proliferation of NK cells</td>
<td>Induces IgE secretion</td>
<td>[61]</td>
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<td>IL-13</td>
<td>(i) Regulates the growth and proliferation of B cells</td>
<td></td>
<td>[62]</td>
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<tr>
<td>IL-14</td>
<td>(i) Stimulates growth, differentiation and survival of cytotoxic T cells (ii) Induces differentiation and proliferation of NK cells</td>
<td>Chemoattractant for certain immune cells expressing the cell surface molecule CD4.</td>
<td>[53]</td>
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<tr>
<td>IL-16</td>
<td>(i) Produced by cytotoxic CD8+ and CD4+ Th1 T cells (ii) Exhibits an overall significant tumor inhibitory action.</td>
<td>(i) Immune-mediated antitumor response (chemoattraction of B cells and NK cells to the lymph nodes) (ii) Angiostatic effect</td>
<td>[1, 7]</td>
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<tr>
<td>TGF-β</td>
<td>Tumor promoting role at the late stages of carcinogenesis</td>
<td>Tumor suppressive role in the beginning of carcinogenesis</td>
<td>[40]</td>
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<tr>
<td>OPN</td>
<td>Implicated in enhanced metastasis and invasion of tumor cells</td>
<td></td>
<td>[68]</td>
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<tr>
<td>CCL2</td>
<td>(i) Induces the recruitment of macrophages (ii) Induces angiogenesis and matrix remodeling (iii) Promotes prostate cancer cell proliferation, migration, invasion, and survival</td>
<td></td>
<td>[69]</td>
<td></td>
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<tr>
<td>CCL21</td>
<td>(i) Immune-mediated antitumor response (chemoattraction of B cells and NK cells to the lymph nodes) (ii) Angiostatic effect</td>
<td></td>
<td>[70, 71]</td>
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a significant role in tumor growth, angiogenesis, epithelial to mesenchymal transition (EMT), and invasiveness [43, 45]. The aforementioned part indicates that the IL-6/IL-8 duet seems to exert both anti- as well as protumorigenic functions. IL-1 proinflammatory cytokine (both α and β forms) is also a SASP component secreted at lower levels compared to IL-6/IL-8 and interestingly IL-1α regulates the signaling network that leads to the expression of the latter duet [46].

4. Cytokine-Mediated Growth Signaling, Replication Stress, and Genomic Instability

Recently it has been proposed that genomic instability is an evolving hallmark of cancer and Hanahan and Weinberg (2011) established that it constitutes an enabling characteristic for the acquisition of the essential functions (hallmarks) of a cancerous cell [6, 47]. Genomic instability is present in most human cancer types and has various forms. The most frequently occurring one is chromosomal instability (CIN) which involves structural and numerical chromosomal changes that occur in cancer cells over time [47]. Another common form is microsatellite instability (MIN or MSI) which is caused by alterations in DNA mismatch repair genes and leads to changes in the number of oligonucleotide repeats in microsatellite sequences [47]. Genomic instability is observed from early stages of cancer development, even before the acquisition of the cell’s cancerous phenotype [48]. CIN is more frequently observed in human cancers compared to MIN and this might be explained by the formation of double-strand breaks (DSBs) in precancerous lesions and cancers according to the oncogene-induced DNA damage model [49].

Recent studies have unveiled the potential effects of enhanced growth signalling in age and/or age-related diseases, such as cancer. The proposed mechanism involves the induction of DNA replication stress which leads to the formation of DNA double-strand breaks (DSBs), favouring genomic instability and tumorigenesis [50, 51]. Indeed, up-regulation of growth-signalling pathways in all eukaryotes has been shown to impact cellular processes leading to increased oxidative DNA damage and replication stress in a correlative manner. DNA replication stress may occur by any mechanism causing slow progression or stalling of DNA replication forks and, as a result, compromise proper DNA replication [50]. The structural characteristics of the replicating DNA strands are greatly responsible for the induction of replication stress-induced DNA damage, as any lesions of the single-strand templates within the unfolded DNA at the sites of the replication forks subsequently cause DSBs. Consequently, the genetic sequence harbouring DSBs is rendered highly susceptible to serious gene rearrangements and genomic instability. Contemporary studies have clearly shown the activation of the replication stress-induced DNA damage response (DDR) pathway at the earliest stages of cancer development underlying its significance in carcinogenesis [48, 52].

Several cytokines exhibit growth factor activity. The most significant cytokines to date designated as growth factors are the following: Epidermal Growth Factor (EGF), Platelet-Derived Growth Factor (PDGF), Fibroblast Growth Factor (FGF), TGF-α, TGF-β, Erythropoietin, Insulin-Like Growth Factor 1 (IGF-1), Insulin-Like Growth Factor 2 (IGF-2), IL-1, IL-2, IL-6, IL-8, TNF-α, TNF-β, INF-γ, and Colony Stimulating Factors (CSFs) (Table 1). Within this context a potential mechanism linking persistent chronic inflammation with carcinogenesis is through the activity of growth-promoting cytokines in the inflamed tissue. It has been previously shown that the injection of adenoviral vectors expressing a cocktail of growth factors (including fibroblast growth factor, stem cell factor, and endothelin-3) in human skin xenografts causes allelic imbalance in common fragile sites (CFs), suggesting the formation of DSBs through replication stress [48]. In accordance with this, the presence of several growth-promoting cytokines within the inflammatory milieu for a prolonged period of time may induce replication stress and subsequent DSBs (Figure 1). In a rat silica model of inflammation-induced lung cancer the presence of the DNA damage response marker γH2AX was observed from early hyperplastic tissues, in bronchiolar hyperplasia, which supports the previous statement [74]. In another study the addition of IGF-1 or IGF-2 in human peripheral blood lymphocytes already incubated with bleomycin further increased the expression of p53 and the rate of chromosome aberrations, suggesting potential implication of DDR activation [75]. Overall, these findings indicate a potential role

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<th>Cytokine</th>
<th>Protumorigenic role</th>
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<th>Unspecified yet role in tumorigenesis</th>
<th>References</th>
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<tbody>
<tr>
<td>CCL16</td>
<td>(i) Suppress antitumor immunity in the tumor microenvironment (ii) Regulates trafficking of immature and maturing immune cells (iii) Promotes angiogenesis (iv) Facilitates metastasis</td>
<td>Augments the cytotoxic activities of effector T cells</td>
<td></td>
<td>[72]</td>
</tr>
<tr>
<td>CXCL12</td>
<td>(i) Suppress antitumor immunity in the tumor microenvironment (ii) Regulates trafficking of immature and maturing immune cells (iii) Promotes angiogenesis (iv) Facilitates metastasis</td>
<td></td>
<td></td>
<td>[73]</td>
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NK cells: Natural Killer cells; IgE: Immunoglobulin E; MHC: Major Histocompatibility Complex; OPN: Osteopontin.

Table 1: Continued.
of cytokines with growth factor activity in the promotion of genomic instability through replication stress-induced DNA damage.

5. Cytokines and Oxidative/Nitrosative Stress

Reactive Oxygen and Nitrogen Species (RONS) are the free radical forms of oxygen and nitrogen, respectively. Free radicals contain one or more unpaired electrons rendering them highly reactive molecular metabolites [76, 77]. RONS are produced by endogenous as well as exogenous sources. Endogenous sources include metabolic reactions, such as electron transport reactions in the mitochondrial respiratory chain, metal reactions, and cells of the innate immune system, such as neutrophils and macrophages, during inflammatory responses [33, 77]. Exogenous sources include atmospheric pollutants, ionizing and nonionizing radiation, several carcinogenic compounds, and metal ions [77].

The most commonly generated Reactive Oxygen Species (ROS) in cells are the superoxide anion (O$_2^-$), hydrogen peroxide (H$_2$O$_2$), and the hydroxyl radical (OH$^-$). The NADPH oxidase is an enzyme that plays the role of an electron donor and generates O$_2^-$ from oxygen in the body [78]. This enzyme can also lead to the production of H$_2$O$_2$ on neutrophil membranes [77]. NADPH oxidase exists in both phagocytes and nonphagocytes and has five known isoforms (NOX 1–5) as well as two reported related enzymes (DUOX 1–2) to date [78]. Nitric oxide (NO*) is a reactive free radical that generates additional Reactive Nitrogen Species (RNS) and is more stable in a hypoxic environment than in normal oxygen tension conditions [33, 77]. NO* is generated by the enzyme nitric oxide synthase (NOS) during the metabolization of L-arginine to citrulline [77, 79]. This enzyme exists in three isoforms in mammalian cells which consist of the neuronal (n)NOS, the endothelial (e)NOS, and the inducible (i)NOS [22, 80]. The nNOS and eNOS isoforms are constitutively expressed and produce low levels of NO*, while iNOS produces high levels of nitric oxide only upon inflammatory stimuli [22, 33]. The nNOS and eNOS isoforms generate NO* with a neurotransmitting and vasodilating role, respectively, while the iNOS isoform produces NO* as a mediator of the inflammatory response [33, 81].

RONS play a dual role in the cell, at low concentrations they are beneficial exhibiting several physiological functions in cellular responses (such as cellular signaling pathways and mitogenic responses), whereas at high concentrations they are detrimental for its integrity [77, 82, 83]. Excessive ROS and RNS production leads to oxidative and nitrosative stress, respectively. In order to counteract the harmful effects of free radicals the cell has evolved several defense mechanisms. These mechanisms involve the action of enzymatic, such as superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and nonenzymatic antioxidants, such as Vitamin C and E, glutathione, carotenoids, and flavonoids [33, 77].

As mentioned earlier, RONS can be released by immune cells as a response to an inflammatory stimulus. Interestingly, during an inflammatory response phagocytic cells can...
directly produce RONS whereas in nonphagocytic cells their production is triggered by proinflammatory cytokines, such as IL-1 and IL-6 [22, 84]. In 1997, it was the first time reported that specific proinflammatory cytokines, IL-1, TNF-α, and IFN-γ, generate ROS in nonphagocytic cells [77, 85]. Later on, it was shown that IFN-γ, TNF-α, and IL-1β promote ROS through the induction of NOX1 in colon epithelial cells [78]. IFN-γ also increases the expression level of the NOX2 isoform of NAPDH oxidase in human macrophages and neutrophils as well as the expression of its DUOX2-related enzyme in airway epithelial cells [78]. The anti-inflammatory cytokine TGF-β induces NOX4 expression in many cell types, such as cardiac fibroblasts, smooth muscle cells, and hepatocytes. IL-4 and IL-13 anti-inflammatory cytokines augment the expression level of DUOX1 enzyme in airway epithelial cells [78, 86–89]. Recently, it was shown that the inflammatory mediator leukotriene B₄ (LTB₄) induces NOX1 in human mast cells [90]. Finally IL-1, TNF-α, and IFN-γ proinflammatory cytokines stimulate the production of iNOS, which is inducibly expressed in macrophages, and thus contribute to the formation of nitric oxide as well as RNS [35, 91]. Overall, both pro- and anti-inflammatory cytokines have been shown to contribute to RNS production through the induction of NAPDH oxidase and iNOS activity. Interestingly, it has been shown that the proinflammatory cytokines seem to play a tumor promoting role while anti-inflammatory cytokines exert an antioncogenic function [22]. The latter leads to the suggestion of a potential link between inflammation, free radical-induced stress, and carcinogenesis.

6. Free Radicals and Inflammatory-Induced Carcinogenesis

Reactive Oxygen and Nitrogen Species (RONS) can be generated during inflammatory responses, and interestingly some inflammation-associated cancers are linked to oxidative and nitrosative stress. Examples include colorectal cancer provoked after active chronic colitis as well as lung, pancreatic, and esophageal cancers provoked after persistent inflammation of the bronchi, pancreas, and oesopagus respectively [33, 92–95]. In the previously mentioned cancer types either excessive free radical production or defective antioxidant mechanisms or both of these were observed.

Inflammation-mediated ROS production can trigger carcinogenesis either directly or indirectly [33]. Direct effects involve the formation of DNA cross-links, single- or double-strand breaks leading to mutations in oncogenes and tumor suppressor genes and ultimately to genomic instability (Figure 1) [33, 77, 84, 96]. An example is the formation of an extremely reactive free radical species during inflammatory response, named peroxynitrite anion (ONOO−), produced by the reaction between the superoxide anion and nitric oxide, which can create DNA fragmentation [77]. Additionally, the hydroxyl radical is known to cause damage to the purine and pyrimidine bases as well as the deoxyribose backbone [77, 97]. The most known DNA damage indicative of oxidative stress is the formation of 8-hydroxydeoxyguanosine (8-OHdG) that is generated by the oxidative attack of OH• in the cell DNA [33]. Free radical production during inflammatory process can indirectly lead to carcinogenesis via ROS-mediated activation of signalling pathways (Figure 1) [33]. An important example is the involvement of oxidative stress in the activation of the transcription factor NF-κB which in turn may display a protumorigenic effect, as mentioned earlier. Low to mild levels may lead to NF-κB expression, since H₂O₂ has been shown to degrade the IkBα subunit, whereas high levels of oxidative stress in the cells may cause inhibition of NF-κB expression [33, 98, 99]. Interestingly, growing evidence supports the fact that ROS act as second messengers in the NF-κB activation through the proinflammatory cytokines TNF and IL-1 [33, 77]. It is also worthwhile mentioning that iNOS can be induced by several stimuli, including cytokines as well as NF-κB transcription factor [22, 100]. Free radicals and cytokines can both either induce or become induced by NF-κB and their generation by tumor cells in inflammation-induced cancers can actually create a positive feedback loop for their own production. The inflammatory microenvironment that develops around the tumors leads to additional production of cytokines and free radicals, which in turn favours the carcinogenesis process [22]. In addition, oxidative stress can cause MIN by reducing the enzymatic activity and expression of the DNA mismatch repair genes mutS homologs 2 and 6 [22, 33]. Free radicals lead to gene silencing of the DNA mismatch repair gene hMLH1 via hypermethylation induced by overexpression of DNA methyltransferases [22, 33].

Cancer development involves three stages, initiation, promotion, and progression, and oxidative stress is involved in all the previous. In the initiation stage, ROS promote DNA damage, such as 8-OHdG formation, that may subsequently lead to gene mutations [33, 77]. During the promotion stage, low levels of oxidative stress can cause modifications in second messenger systems and promote cell division and proliferation [33, 77]. Finally in the progression stage, ROS can create additional genetic alterations fuelling cancer cells with further evolutionary advantages [33, 77]. Overall, the action of RONS is dosedependent within a particular cell context often with tumor-promoting activity in low levels, whereas in high levels they may raise the antitumor barriers, namely, apoptosis and senescence [33].

7. Future Perspectives

Cytokines display pleiotropic actions ranging from tumor-protective to tumor-promoting activity in a spatial and temporal manner. The fact that certain cytokines display growth factor activity as well as the ability to produce RONS suggests that they may promote genomic instability in chronic inflammatory conditions. This is particularly significant taking into account the central role of cytokines as mediators of inflammation, a knowledge that can be exploited in future cancer therapeutic strategies. IL-2 and IL-15 are currently tested for their therapeutic potential in cancer [53]. It has been shown that IL-15 plays an important role in the antitumor efficacy of combination therapy with Imatinib
Mesylate (IM), a tyrosine kinase inhibitor, and IL-2 in a mouse lung metastasis model, inducing a CCL2-dependent chemotraction of IFN-producing killer dendritic cells (IKDCs) in the tumor microenvironment [101]. The latter mouse lung metastasis model, inducing a CCL2-dependent Mesylate (IM), a tyrosine kinase inhibitor, and IL-2 in a another paradigm of translation of molecular biology to sarcomas and TRAIL-sensitive cancers. CCL2 serves as phase I clinical trial targeting IM-resistant gastrointestinal finding has been exploited therapeutically by launching neutralizing antibody against CCL2 is under clinical trials in prostate cancer. Conclusively, elucidation of the molecular mechanisms implicated in tumor-host interactions may provide new insight in understanding tumor development as well as provide additional future prospects for more effective and targeted cancer therapy and prevention.

Acknowledgments

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References


Oral Lichen Planus as a Preneoplastic Inflammatory Model

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1. Introduction

Oral lichen planus (OLP) is a chronic inflammatory oral condition of unknown aetiology characterized by T-cell-mediated chronic immune response and abnormal epithelial keratinization cycle [1]. The OLP lesions may coexist with cutaneous and genital lesions, or may be the only disease manifestations [2]. The epidemiology of OLP is not easy to calculate with reported incidence ranging between 1-2% of the general population. Recent meta-analysis calculated a 1.27% incidence in the general population [3]. The OLP lesions are consistently more persistent than the dermal lesions and have been reported to carry a risk of malignant transformation to oral squamous cell carcinoma (OSCC) of 1-2% (reported range of malignant transformation 0–12.5%) [4]. Clinically, OLP appears more commonly with the classic reticular form, which results from coalition of papules and may be asymptomatic or may cause mild discomfort. Erythema, erosions, and ulceration could also appear and these are the most painful OLP manifestations, while if the lesions become chronic they may become hyperplastic or atrophic [4]. The lesions of OLP tend to present symmetrically and bilaterally especially in the buccal mucosa [5]. Histological examination of OLP reveals, dense inflammatory infiltrate in the upper lamina propria, mainly consisting of T-cells, liquefaction degeneration of basal keratinocytes and basal membrane hyperkeratosis or atrophy of the keratin layer [6, 7]. The pathogenesis of OLP is very complex and involves possible antigen presentation by the oral keratinocytes that could be either of an exogenous or an endogenous origin [8–10]. This antigenic trigger is accompanied by a mixed inflammatory response comprising mainly T-cells, macrophages, and mast cells, as well as the associated cytokines and cytotoxic molecules [4, 8–10]. Officially, the World Health Organisation (WHO) classifies OLP as a “potentially malignant disorder” with unspecified malignant transformation risk and suggests that OLP patients should be under close monitoring [11]. The possible premalignant
nature of OLP has been the subject of numerous studies and great controversies [4, 5]. Treatment of OLP is remarkably unsatisfying; topical steroids are the first treatment choice and systemic corticosteroids and immunosuppressants are the second line agents, but none of them can result in significant long-term disease control [12]. Severe erosive disease leaving mucosal atrophy and requiring systemic treatment is reported to carry the highest risk of malignant transformation [13]. There is no definite malignant transformation mechanism identified in OLP. The current hypothesis is that chronic stimulation from the inflammatory and stromal cells is providing the signals that are causing epithelial cells to derange their growth control and in cooperation with oxidative stress, from oxidative and nitrative products, it provokes DNA damage resulting in neoplastic changes [4, 14–17] (Figure 1). Recently, OLP has been proposed to be an ideal model of inflammation induced cancer [18].

The advances in molecular information on this pathologic condition have shed new light on the complex pathogenesis of OSCC arising in OLP and this article is an attempt to review the currently available data.

2. Cell Cycle Control in Oral Lichen Planus

Apoptosis of basal keratinocytes, caused by the activity of cytotoxic T-cells, could be a possible explanation for one of the histopathologic hallmarks of OLP that is the vacuolar degeneration of basal membrane [8]. This is also supported by several molecular studies demonstrating the presence of apoptotic signals in OLP [10, 19, 20].

Nevertheless, if apoptosis was the main cellular event, then all cases of untreated OLP would end up with severe and extensive oral mucosa erosions [21]. However, this is not the case in the majority of OLP, as the most common clinical form of OLP is reticular lichen planus, while the erosive forms usually are limited in one or two oral sites [4, 5]. Therefore, a counterbalancing mechanism is expected as a response from the oral epithelium to maintain its integrity. In fact, several molecular studies indicated evidence of increased cellular turnover rate, in the form of increased cellular proliferation, in epithelial cells of oral lichen planus [21–24]. In addition, other authors have demonstrated mixed patterns of both apoptosis and increased cellular proliferation occurring simultaneously [25–27].

Even more, González et al. suggested that possibly epithelial cells in OLP respond to the inflammatory chronic attack by exhibiting a senescent phenotype instead of apoptosis. This hypothesis was based on the observed positive p21WAF1 expression in OLP, which is indicative of cell cycle arrest and possibly of senescence [28].

Cell cycle arrest helps in maintaining tissue integrity and facilitating DNA repair mechanisms, but at the same time entry into senescence could favor malignant transformation [29–31]. As the authors note too, positive p21WAF1 is only indicative of senescence, in contrast to the most established marker of senescence which is SA-beta gal staining [28]. Nevertheless, given that this staining method is not suitable for paraffin embedded tissues, [32] which is the most widely available material for OLP studies, data after applying this method are lacking.

In a similar hypothetical model, Poomsawat et al., considered their observations of increased p16INK4A and cdk4 expression in OLP as evidence of a precancerous OLP process [33]. In a contradictory study, Montebugnoli et al. did not find significant differences in p16INK4A expression in OLP and nonspecific oral inflammation and interpreted the p16INK4A expression only as a sign of inflammation [34].

3. The Role of p53 in OLP

Inactivation of p53 is a frequent phenomenon in OSCC. This is caused by mutations, presence of HPV virus and other molecular alteration occurring in the p53 pathway [35]. The studies investigating the expression of p53 in OLP...
have been recently reviewed by Ebrahimi et al. [36]. In their vast majority, they included immunohistochemistry-based reports and their results varied significantly, with reported expression percentages ranging from 0–100%. Nevertheless, most of them found significantly higher expression in OLP than in normal oral mucosa [36]. As p53 expression has been identified as a response to DNA damage, [37] the identification of p53 in OLP tissue is interpreted as an indication of precancerous potential by some researchers [24, 38]. In support to this concept, Chaayarit et al. showed an i-NOS-dependent DNA damage and p53 elevated expression in OLP patients [39]. Another concept is that the high expression of p53 in OLP is a result of the higher cellular proliferation [22, 40]. To prove that p53 expression in OLP is not just a result of the inflammatory process, Safadi et al. [38] compared the immunohistochemical expression of p53 and of its downstream effector p21WAF1 between OLP and other inflammatory oral conditions and found significantly higher expression in OLP [38]. What is still unclear is the underlying mechanism that drives p53 expression in a significant percentage of OLP cases, but as p53 expression in OLP is comparable to that observed in dysplastic oral lesions, it is considered as a sign of malignant potential [36].

At this point, it is tempting to speculate that OLP as an inflammatory condition, along with the accompanying oxidative stress, probably induces a genotoxic stress. In addition, the high proliferation rates reported for the oral epithelium turnover in OLP may also create a replication stress. Such conditions should activate the DNA damage response (DDR) checkpoint [41, 42]. In turn, this pathway should elicit the p53-mediated antitumor barriers of apoptosis and senescence. Continuous activation of this checkpoint will eventually surpass the cell repair capacity predicting the emergence of genomic instability and finally selective p53 inactivation. Consecutively, this would result in the progression to malignancy. Nevertheless, this scenario requires experimental validation, despite the presence of experimental evidence compatible with it.

4. Chromosomal Instability in OLP

To verify the OLP malignant potential hypothesis, genetic alterations observed in epithelial cancers have also been studied in OLP. In 1997, Zhang et al. used microsatellite analysis to investigate loss of heterozygosity (LOH) at loci 3p, 9p, and 17p, which is frequently observed in oral cancers [43]. Despite they detected LOH, their results showed no different frequencies from the reactive irritation (benign inflammation). Nevertheless, while this result did not support OLP as a lesion at risk for malignant transformation, the authors could not exclude that OLP may undergo malignant transformation through other genetic pathways [43]. Following these results, the same authors performed the same loci analysis in dysplastic lesions in OLP patients and their results showed comparable rates of allelic loss with those observed in epithelial dysplasia even for cases of mild dysplasia [44]. From this finding they concluded that dysplasia observed in OLP cases is possibly an independent risk factor for malignant transformation and underlined that very diligent clinical and pathologic approach should be applied in the case of OLP biopsies [44]. Similar results and conclusions especially for LOH in chromosome 9 in OLP-associated dysplasia were reported by Kim et al. with the use of chromosomal in situ hybridization [45]. On the other hand, in a more recent study using laser capture microdissection and microsatellite analysis to identify LOH, the results were similar in benign lesions and OLP samples weakening the concept of malignant OLP potential, but these authors also emphasize on careful histopathologic examination of OLP samples [46]. Of note, all data available from LOH analyses are confined only to chromosomes 3, 9, and 17 [43–46]. To the best of our knowledge, genome-wide analyses in large cohorts of OLP are still missing.

Changes in DNA ploidy are also an indication of malignancy. DNA ploidy studies in OLP have demonstrated that some atrophic lesions may be found aneuploid, but the results are not indicative of a potentially malignant process [47–49]. Abnormal karyotypes and chromosomal alterations associated with p53 expression have also been detected in OLP, but the data are small to allow safe conclusions [50].

5. Matrix Metalloproteinases (MMPs) and OLP

Sutinen et al. were among the first to investigate the expression of MMPs and their inhibitors TIMPs in clinical samples with OSCC, OLP, dysplasia, lymph node, metastases, and normal oral mucosa [51]. Though their findings showed significantly higher expression in OSCC in comparison to the other lesions, they first noted a weak MMP 1 and 2 expression in some OLP cases [51]. Subsequently, Zhou et al. reported increased expression of MMP 1–3 in the epithelial OLP cells and MMP-9 in the OLP inflammatory infiltrating cells, but not the TIMPs, and suggested a role of MMPs in the basement membrane disruption, which possibly enables intraepithelial inflammatory cell migration [52].

The role of MMPs in OLP was initially associated with apoptosis of epithelial cells and the level of inflammation [53]. Transforming growth factor beta (TGF-β) and the bone morphogenic protein-4 (BPM-4) were suggested as promoting signals for the upregulation of the MMPs [53, 54]. Chen et al., studied MMPs, TIMPs and TGF-b in OSCC that developed from previous OLP and found constant expression with levels comparable to those detected in atrophic OLP, which is the form of OLP reported to have the higher malignant potential [55]. They concluded that their findings are suggestive of the role MMPs have in the malignant transformation in OLP [55]. More recently, Tsai et al. detected elevated MMP-2 levels both in situ, and in peripheral blood of the same patients, interpreting their findings as indices of systemic inflammation, in OLP [56].

6. The Role of NF-KappaB and Associated Cytokines (IL-1α, IL-6, IL-8, TNF)

The transcription factor Nuclear Factor kappa betta (NF-kappaB) has been described as a major molecule associating chronic inflammation and cancer mainly by inhibiting
apoptosis, promoting cellular proliferation and favoring metastatic phenotypes [57]. The expression of NF-kappaB has been reported higher in OLP than in cutaneous lichen planus (CLP), a fact that is considered consistent with the more persistent inflammation observed in OLP in comparison to CLP [58]. In support to the above, the levels of NF-kappaB associated cytokines (IL-1α, IL-6, IL-8, TNF) have been found increased in whole unstimulated saliva and other oral fluids of OLP patients [59–61] and also in OSCC patients [60]. These observations are suggestive for a role of NF-kappaB and of the associated cytokines in the inflammatory process of OLP and possibly also in the malignant transformation of OLP [59–61].

TNF is one of the most studied cytokines linking chronic inflammation and cancer by inducing neoplastic cellular phenotypes, and angiogenesis [62]. TNF involvement in the pathogenesis of OLP has been proposed for more than 15 years ago [63]. Since then, several studies demonstrated findings supporting the TNF involvement in OLP pathogenesis. These include TNF genetic polymorphisms with OLP susceptibility, [64–67] elevated serum and saliva TNF levels in patients with OLP, [68–71] and in situ detection of TNF in OLP epithelium [72, 73]. Its role is also supported by the favorable results of anti-TNF agents in patients with OLP [74, 75].

IL-6 expression in serum and saliva of OLP patients [76] is considered indicative of a Th2 cellular involvement in OLP, [77, 78] a fact that was underestimated initially in the pathogenesis of OLP [8]. Similarly, IL-6 has been associated with promoting colon cancer development in inflammatory bowel diseases [79, 80]. Furthermore, IL-6 and IL-8 expression is associated with the senescence phenotype and has been suggested that they promote senescence-related growth arrest [81].

7. Hepatitis C Virus (HCV) Infection and OLP Malignant Potential

HCV infection has been associated with OLP pathogenesis in certain ethnic populations, especially in the Mediterranean area [82]. HCV infection is a well-documented risk factor for hepatocellular carcinoma development [83]. Also, chronic HCV infection has been implicated with other malignancies like cholangiocarcinoma and lymphomas [84]. The pathogenetic mechanisms that connect OLP and HCV were based on the findings that circulating antibodies against the oral epithelium were identified in OLP patients with HCV infection, [85] and that OLP mediating cytokines are triggered by HCV infection [86]. A study in Japanese OLP patients identified HCV RNA in oral lesions and serum from OLP and OSCC patients and concluded that it may be involved in the pathogenesis of OSCC [87]. In contrast, in a study of oral epithelial dysplasia and HCV infection in British population, no such association was observed [88]. The association of HCV infection and OLP development in certain ethnic groups may be related to HLA subclasses presence, and though weak, some evidence exists to correlate these diseases suggesting further investigation [89]. Nevertheless, up to now no strong evidence exists so far to indicate a possible strong association of HCV infection with OLP progression to OSCC.

8. Similarities between Inflammatory Bowel Diseases (IBD) Associated Colorectal Carcinomas and OLP Associated OSCC

Inflammatory bowel diseases, ulcerative colitis (UC), and Crohn’s disease (CD) are complicated with colorectal carcinomas in a percentage rate of 7–14% for UC in a 25 year time frame, and a 2.9% cumulative risk for CD in 10 years [90, 91].

Patients who develop colorectal carcinomas in IBD may present with multiple sites of cancer and areas of dysplasia in the same way that patients with OLP-associated OSCC may develop new primary tumors and dysplastic lesions in multiple oral sites [92–95]. T-cells and apoptotic mechanisms have an important role, both in OLP and IBD pathogenesis [96, 97].

Recently, the role of the neuronal axon guidance molecule netrin-1 and its receptors (DCC, UNC5H) have been discovered to play a pivotal role in progression of IBD to colon adenocarcinoma, [98, 99] and its expression is probably upregulated through NF-kappaB [100]. This molecule has not been investigated in OLP and could constitute a possible link between chronic OLP inflammation and cancer progression, a hypothesis that we currently investigate.

It is possible that both IBD and OLP, as chronic inflammatory conditions, provide the basis for the establishment of early preneoplastic lesions. These in turn, under the appropriate conditions, may further develop by progressing to malignant stages. The fact that, in contrast to IBD, OLP has less percentage of malignant potential is not against this model as IBD is common in younger adults while OLP is a disease mainly of post menopausal women. Thus, the time frame for malignant development is wider in IBD in contrast to OLP.

9. Future Prospects

It is clear that most of the studies so far have shown indicative results of a precancerous OLP nature. Studies that will include investigation of other unexplored pathways, like the DDR one, and in larger OLP cohorts, especially including all the spectrum of lesions up to full-blown cancer from the same patient, are a prerequisite. A series of evidence like the oxidative stress due to chronic inflammation, the potential replication stress as exemplified by the observed high cellular proliferation, the increased p53 expression, and the genomic instability in OLP are suggesting that DNA damage is taking place in OLP. The hypothesis that could fit in OLP carcinogenesis, based on the available data, is that p53 upregulation in response to continuous oxidative and replicative DNA damage, protects the OLP-affected cells from a malignant potential through activation of the cell cycle arrest, apoptosis and/or senescence. Nevertheless, sustained DDR activation will eventually overwhelm the cellular repair capacity. When this repair mechanism exceeds
its potential, genomic instability will gradually accumulate, leading eventually to mutation fixation in critical genes. As a result the antitumor barriers will gradually diminish and dysplastic changes as well as further ones may occur.

There are still other important questions to consider: Which is/are the main signals that could promote toward cancer? What kind of treatment modalities should be applied? Could new treatment with new biologic agents (e.g., anti-TNF, anti-IL6 receptors) alter the cancer risk in OLP patients? The development of accurate OLP animal models, which currently lack, may also prove to be important tools in the molecular deciphering of both OLP and its progression to cancer. More research is required to acquire a full view of the precancerous nature of OLP and to be able to determine subclasses of OLP patients at increased risk of malignant transformation.

10. Conclusion

All the findings so far are indicative that the OLP is a preneoplastic inflammatory model. The fact that OLP lesions are found in an open cavity, such as the mouth, that is, accessible to regular monitoring and biopsy, is feasible without complications and high cost, render OLP an ideal disease to study the relationship between chronic inflammation and cancer. The focus should be on finding markers that delimit the patients at risk of OSCC progression.

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References


Research Article

Detection of Herplex Simplex Virus-1 and -2 in Cardiac Myxomas

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The etiology of sporadic cardiac myxomas remains elusive. The tendency for these lesions to recur following resection, their immunopathological characteristics, along with their histological and molecular profile, may implicate the presence of an infective agent in this type of tumor. In this study, we investigated the presence of herpes simplex virus (HSV) DNA in a cohort of cardiac myxomas in a tertiary referral centre. Twenty-nine formalin-fixed paraffin-embedded (FFPE) sporadic cardiac myxomas were obtained, 17 of which were shown to be informative. These were compared to 19 macroscopically and microscopically normal heart tissue specimens. The detection of HSV-1 and -2 genomic sequences was achieved with the use of a combined nested PCR-Restriction Fragment Length Polymorphism methodology. The presence of HSV-1 and/or -2 DNA was demonstrated in 6 of 17 (35%) informative sporadic cardiac myxomas, whereas no HSV DNA was detected in normal heart tissues ($P < 0.01$). The existence of HSV-1/2 DNA in sporadic cardiac myxomas, along with its absence from normal heart tissues, reinforces the possibility that HSV infection might be involved in the development of these lesions. Our findings raise the point of anti-HSV medication postsurgically with a potential benefit in reducing the rate of recurrences.

1. Introduction

Primary heart neoplasms are rare and encountered in approximately 0.056–0.02% of autopsy series. These affect patients of all ages, with the vast majority being benign (75–80%), and with the females more often affected than males. Myxoma is the commonest primary cardiac neoplasm with a malignant potential, accounting for approximately 75% of all cases [1], having an annual incidence of about 1/10^6 and a recurrence rate of 2-3% [2, 3]. Some cases are familial and appear to have an autosomal dominant transmission [4]. These are frequently single, affecting mainly the atrial fossa ovalis in the left atrium [1] as part of the Carney complex, which has been found to be associated with the germline mutation PRKARIA encoding Protein Kinase A Regulatory subunit type 1A. Only familiar forms of myxomas have been associated with the mutation; sporadic cases of myxomas have no association with it [4, 5]. Sporadic myxomas, represent the majority of the diagnosed myxomas and their etiology remains elusive. Although recurrences of myxomas have mostly been attributed to incomplete excision, this explanation does not account for recurrences at distant sites within the atria [6].

Various indications point towards the involvement of Herpes Simplex Viruses (HSV) in sporadic cardiac myxoma pathogenesis. The endocardium of the atrial septum, where the atrial myxoma mainly originates from, is rich in sensory nerves. In turn, myxoma cells appear to be derived from endocardial sensory nerve tissue [7]. The life cycle of HSV is characterized by latency in sensory or autonomic ganglia...
that can be maintained for life in the host. Periodically, the virus can be reactivated by stimuli causing either viral shedding or recurrent infection of the affected nerve [8]. In addition, a report in Chinese patients has suggested HSV-1 presence in sporadic atrial myxomas [9]. Our aim was to examine whether HSV-1, 2 DNA could be detected in a cohort of patients with cardiac myxomas more frequently than in normal control hearts.

2. Materials and Methods

Twenty-nine tissue specimens originating from patients with typical clinicopathological features of sporadic cardiac myxoma were examined. The tissue specimens were obtained from Onassis Cardiac Surgery Centre (1996–2003) from patients admitted for surgical removal of the lesion. Tissue specimens from 19 macroscopically and microscopically normal hearts, provided by the forensic department of the university, served as controls. Specific care was taken so as the material from the normal controls was taken from the atria. The control specimens were matched for age and sex with the myxoma specimens. All tissues were formalin-fixed, paraffin-embedded. Use of the myxoma material followed written permission by the subjects, while the local Ethics Committee approved further experimentation. The study was performed in accordance with the requirements of the revised (1983) Helsinki Declaration of 1975. Whole blood from previously tested HSV-1/2-infected patients was used as positive control. Histopathological evaluation was performed by hematoxylin and eosin staining.

DNA extraction was performed according to the conventional phenol/chloroform protocol with slight modifications [10]. The IFN-γ house-keeping gene was used to assess the integrity and yield of the extracted DNA [11]. The primers used in the first-round amplification reaction are the following: 5'-TGCTCTTACAACAAAGTC-3’ and 5’-CGGTGCTCCAGGATAA-3’ with annealing temperature 55°C and respective product size 200 bp. In the second-round amplification reaction the following primers were employed: 5’-ATCCGAAAGCAAGCCCGCTG-3’ and 5’-CTCAGTCCAGTCGTATCTTC-3’ (reverse) with annealing temperature 60°C and corresponding product size 142 bp. They are based on those reported for HSV glycoprotein D gene (GenBank accession numbers: X14112 (HSV-1), Z86999 (HSV-2)) [12]. PCR reactions were performed as previously described [11]. The primers for Interferon γ (IFNγ) are the following: 5’-CTCTTCTTCTCCAGATGT-3’ and 5’-CTGGGATGCTCTTCAGACCTCG-3’ with annealing temperature 57°C and respective product size 151 bp.

Restriction Fragment Length Polymorphism (RFLP) analysis was performed on HSV-positive nested-PCR products, digested with the restriction enzyme MspI (New England Biolabs) at 37°C for 4 h. RFLP digests were size-fractionated by electrophoresis on 7.5%-polyacrylamide gel, stained with ethidium bromide and photographed under UV light.

Data are expressed as mean ± 1 standard deviation (S.D.) for continuous variables and as frequency (percentage %) for categorical data. The normality of the distributions was assessed with Kolmogorov-Smirnov test and graphical methods. Comparisons of continuous variables were performed using Mann-Whitney’s U nonparametric test. Categorical data were compared by Fisher’s exact test. The necessary number of control samples was determined by power analysis (G*Power 3, Universität Kiel, Germany). Differences were considered to be statistically significant if the null hypothesis could be rejected with >95% confidence (P < 0.05).

3. Results

The demographic characteristics of the myxoma patients (all and informative cases) and the normal controls are depicted in Table 1. Twenty patients presented with various clinical manifestation (strokes, pulmonary embolization), while for the remaining 9 patients the myxoma was an incidental finding on cardiac ultrasound. The myxoma was excised from the right atrium in 22 patients (75.9%) from the left atrium in 2 patients (6.9%), while in 5 (17.2%) patients the lesion was excised from the right ventricle. Histological evaluation clearly exhibited the characteristic gel-like stroma of mucopolysaccharides on hematoxylin and eosin staining (Figure 1(a)) [13]. In this cohort of specimens, 17/29 cases produced appropriate DNA yields for further HSV detection. A clear 142-bp band corresponding to both HSV-1 and HSV-2 genomes was obtained following PCR amplification in 6 myxoma cases, accounting for 35.3% of the sample (Figure 1(b)). In contrast, HSV DNA was absent from the 19 normal heart tissues tested (P < 0.01) (Figure 2). Five of the infected cases originated from female patients from the left atrial cavity, and one specimen was removed from the right atrial chamber of a male patient. RFLP analysis allowed the identification of the infectious viral type in 4/6 HSV-positive cases (Figure 2). In fact, two atrial myxomas were found to harbor HSV-1, one was infected by HSV-2, while both viruses were present in an additional case (Figure 3).

4. Discussion

This study has shown that HSV DNA is detected significantly more frequently in cardiac myxomas than in their normal counterparts. The demonstration of HSV-1 infection lies in accordance with a study performed on the Chinese population, which reported on the occurrence of the virus in a subset of sporadic cardiac myxomas [9]. The authors found that the examination of the viral DNA is more sensitive than
The immunohistochemical evaluation of HSV-1 in cardiac myxomas, which may provide an explanation for the findings of a recent study showing no association between HSV and cardiac myxomas [14]. The present study was adequately powered to detect statistical differences between cardiac myxomas and normal hearts regarding the presence of HSV. In addition, the detection of HSV-2 as the infectious agent in two myxoma cases reflects a novel finding. Two others members of Herpesviridae family have been long ago established as oncogenic agents, Epstein-Barr virus (EBV) and Human Herpes Virus type 8 (HHV-8) [15]. EBV is associated with the development of Burkitt’s lymphoma, Hodgkin’s lymphoma (B-cell), and nasopharyngeal carcinoma, and HHV-8 is having a causal role for the development of sarcoma Kaposi.

Common clinical manifestations of myxomas are strokes, peripheral or pulmonary embolization, fever, weight loss, high sedimentation rate, anemia, and leucocytosis [1]. At an immunological level, a CD8+ cellular infiltrate and elevated IL-6 levels have been observed [9, 16, 17]. Additionally, the molecular profile commonly associated with neoplastic disease, such as mutations in gatekeeper genes, is absent [18]. It is likely that, in certain susceptible individuals, the development of cardiac myxoma is the result of an intense inflammatory process, secondary to an inciting agent. In turn persistent inflammation increases DNA mutations and overall genomic instability favoring
neoplastic transformation [19]. It has been well established that chronic inflammation through several inflammatory mediators including cytokines, reactive oxygen, and nitrogen species induce genetic instability, a hallmark of cancer [20]. Alternatively, and not mutually exclusively, the infectious agent may disrupt critical signaling pathways implicated in cell control. Concerning Herpesviridae family, both EBV and HHV-8 target crucial components of cellular machinery including NFkB and p53 as well as the Wnt pathway through the stabilization of b-catenin [15]. The latter is achieved by the production of certain viral proteins which abrogate the ubiquitination of b-catenin. Interestingly, HSV-1 encodes UL-36, a protein with deubiquitinating activity [21]. The examination of the effect of UL-36 in b-catenin stabilization remains an attractive issue that may shed light on the potential oncogenic properties of HSV-1. Altogether, the above may provide an explanation for the presence of chromosomal aberrations including aneuploidy in a subset of cardiac myxomas [22, 23].

Mucopolysaccharides found in cardiac myxomas have been implicated as receptors of HSV particles during infection [24]. The fact that 6 cardiac myxoma specimens were found to be HSV positive in contrast to the complete absence of HSV in the control group despite the reported prevalence of these viruses in the general population [25] renders the possibility of endocardial infection to be a random event rather unlikely.

The role of HSVs in the pathogenesis of cardiac myxoma is still unclear. Whether the viruses are among the inciting agents for the development of the lesion or simply represent opportunistic pathogens with a tropism for myocardatous endocardium remains to be answered [9]. One may assume that cardiac myxomas arise as a direct consequence of lytic-HSV infection. The viral life cycle is characterized by latency in sensory or autonomic ganglia. Periodically, the virus can reproduce resulting in the stabilization of b-catenin [15]. The latter is achieved by the production of certain viral proteins which abrogate the ubiquitination of b-catenin. Interestingly, HSV-1 encodes UL-36, a protein with deubiquitinating activity [21]. The examination of the effect of UL-36 in b-catenin stabilization remains an attractive issue that may shed light on the potential oncogenic properties of HSV-1. Altogether, the above may provide an explanation for the presence of chromosomal aberrations including aneuploidy in a subset of cardiac myxomas [22, 23].

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Of particular interest is the recognition of HSV-2 as a potential cardiovascular pathogen. The virus has been implicated in coronary artery disease and carotid atherosclerosis [30]. Moreover, infection by HSV-2 seems to convey an increased risk for cardiovascular death and myocardial infarction [31].

This study has a number of limitations. Its major limitation is the small sample size. However, cardiac myxomas are extremely rare and the investigators powered the study enough to detect significant differences, if present. In addition, the cardiac samples that served as controls were received from apparently healthy hearts. Even if the investigators made sure that no cardiac involvement had
led to the death of the subjects and the hearts were found to be microscopically healthy, the possibility of a possible underlying cardiac disease cannot be excluded.

Since myxomas are clinically significant lesions, with potential life-threatening sequelae that affect patients of all ages and the only available treatment is complete resection of the tumor, the identification of possible underlying treatable causes is extremely important. Our work supports the occurrence of HSV in a subset of sporadic cardiac myxomas. While further studies are needed to clarify the role of HSV1 in cardiac myxoma pathogenesis, it is conceivable to trial suppressive anti-HSV drugs, such as acyclovir, after surgery in the HSV-positive patients, in order to avoid a possible recurrence of sporadic cardiac myxomas.

**Authors’ Contribution**

I. S. Pateras and K. Evangelou contributed equally to this paper.

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**References**


Review Article

Asbestos-Induced Cellular and Molecular Alteration of Immunocompetent Cells and Their Relationship with Chronic Inflammation and Carcinogenesis

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Asbestos causes lung fibrosis known as asbestosis as well as cancers such as malignant mesothelioma and lung cancer. Asbestos is a mineral silicate containing iron, magnesium, and calcium with a core of SiO2. The immunological effect of silica, SiO2, involves the dysregulation of autoimmunity because of the complications of autoimmune diseases found in silicosis. Asbestos can therefore cause alteration of immunocompetent cells to result in a decline of tumor immunity. Additionally, due to its physical characteristics, asbestos fibers remain in the lung, regional lymph nodes, and the pleural cavity, particularly at the opening sites of lymphatic vessels. Asbestos can induce chronic inflammation in these areas due to the production of reactive oxygen/nitrogen species. As a consequence, immunocompetent cells can have their cellular and molecular features altered by chronic and recurrent encounters with asbestos fibers, and there may be modification by the surrounding inflammation, all of which eventually lead to decreased tumor immunity. In this paper, the brief results of our investigation regarding reduction of tumor immunity of immunocompetent cells exposed to asbestos in vitro are discussed, as are our findings concerned with an investigation of chronic inflammation and analyses of peripheral blood samples derived from patients with pleural plaque and mesothelioma that have been exposed to asbestos.

1. Introduction

Asbestos causes lung fibrosis known as asbestosis, a few benign pleural diseases such as pleural plaque and effusion, and malignant diseases such as mesothelioma and lung cancers [1–5]. Furthermore, cancers in other organs such as the gastrointestinal tract, larynx, kidney, liver, pancreas, ovary, and hematopoietic systems show a high prevalence in asbestos-inhaled people [6–9]. This issue has been tackled as a major medical and social problem throughout the world, especially since asbestos is very useful in various industries for its mineral characteristics such as resistance to heat, cold, chemicals, cheapness, easiness to obtain and weave, and so on [8, 9].

In Japan, the quantity of asbestos produced has increased since the mid-1950s and reached a peak in 1974 (over 350,000 tons) [10–12]. The peak level of usage continued until the late 1980s in construction, car, and other industries. In the summer of 2005, Kubota Corporation, which mainly
used asbestos to make water pipes in Amagasaki City, Hyogo Prefecture, Japan, acknowledged the prevalence of asbestos-related diseases among their workers, including several patients living near Kubota’s asbestos-handling factory in Amagasaki City [10–12]. Citizens in Japan were suddenly made aware that asbestos causes malignancies in asbestos-handling workers and in residents living near these factories. They were informed that mesothelioma is difficult to diagnose and cure and were angered that workers and neighborhood residents had not been notified that these factories had been handling this silent bomb, asbestos [10–12].

To reduce the anxieties of the Japanese people, epidemiological analyses commenced regarding the Amagasaki area, and clinical and basic research was conducted on the biological effects of asbestos and the early detection of mesothelioma. It is in this context that the authors became involved in the project “Comprehensive Approach on Asbestos-Related Diseases”, supported by the “Special Coordination Funds for Promoting Science and Technology” (headed by Dr. Takemi Otsuki, Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan) from 2006 to 2010. In this project, a case and clinical specimen registration system was established. A feasible clinical trial was established and involved a combined trimodality therapy using anticancer chemotherapy with cisplatin and pemetrexed, followed by extrapleural pneumonectomy and postoperative radiation therapy for early-stage mesothelioma patients [13, 14]. Furthermore, early detection procedures were developed using serum or pleural effusion (PE) mesothelioma patients [13, 14]. Procedures for detection of circulating mesothelioma cells and circulating epithelial cells using peripheral blood [15–19].

For the basic research, the project “Comprehensive Approach on Asbestos-Related Diseases” included three subgroups: (1) analyses of cellular and molecular characteristics using mesothelioma cell lines, (2) an investigation of asbestos-induced carcinogenesis using an animal model, and (3) a study of the immunological effects of silica/asbestos.

The basic research project (3) was performed by us, and in this paper, we introduce our viewpoint that asbestos may cause alteration of immunocompetent cells resulting in chronic inflammation as well as tumor development.

2. Immunological Effects of the Mineral Silicate on Asbestos and Silica

Asbestos is a mineral silicate containing iron, magnesium, and calcium, with a core of “Si” and “O” [6, 7]. Individuals exposed to silica and asbestos develop lung fibrosis known as silicosis and asbestosis, respectively [1–5, 20]. Silicosis is a form of occupational lung disease caused by inhalation of crystalline silica dust, and is marked by inflammation and scarring in the form of nodular lesions in the upper lobes of the lungs [20]. However, asbestos develops in patients who inhale a relatively high dose of asbestos (compared with patients with pleural lesions such as mesothelioma and plaque). In addition, the pathology of asbestosis differs from that of silicosis [1–5]. Asbestosis involves the scarring of lung tissue (around the terminal bronchioles and alveolar ducts). There are two types of fibers: amphibole (thin and straight) and serpentine (curved). The former are primarily responsible for human disease as they are able to penetrate deeply into the lungs. When such fibers reach the alveoli in the lung, where oxygen is transferred into the blood, the foreign bodies (asbestos fibers) cause the activation of the lung’s local immune system and provoke an inflammatory reaction [1–5]. This inflammatory reaction can be described as chronic rather than acute, slow ongoing activation of the immune system in an attempt to eliminate the foreign fibers. Macrophages phagocytose the fibers and stimulate fibroblasts to deposit connective tissue. Due to the natural resistance of asbestos fibers to digestion, the macrophage dies off, releasing cytokines and attracting further lung macrophages and fibroblastic cells to lay down fibrous tissue, which eventually forms a fibrous mass [21, 22]. The result is interstitial fibrosis. The fibrotic scar tissue causes alveolar walls to thicken, which reduces elasticity and gas diffusion, reducing oxygen transfer to blood as well as the removing of carbon dioxide.

Furthermore, the complications in silicosis and in people who have inhaled asbestos are also different. Patients with silicosis often present with complications involving autoimmune diseases such as rheumatoid arthritis (known as Caplan’s syndrome) [23–25], systemic sclerosis [26, 27], systemic lupus erythematosus [28, 29], and antineutrophil cytoplasmic antibody (ANCA)-related vasculitis/nephritis [30–32]. Although these have been considered adjuvant effects of silica, we have assumed that silica may influence circulating immunocompetent cells, particularly T lymphocytes, and have reported that silica can activate CD4+25 + FoxP3 (forkhead box P3) + regulatory T cells (Treg) and responder T cells (Tresp) [33–38]. These activations induce overexpression of CD95/Fas in Treg, resulting in early loss and contamination of activated Tresp, which express CD25 as the marker for activation into a peripheral CD4+25+ subpopulation of T cells. These phenomena seem to induce reduced regulation of autoimmunity [37, 38]. Regarding silica-induced fibrosis (lung and skin), the idea proposed previously [27] is that silica affect alveolar macrophages, endothelial cells, and fibroblasts to modify cytokine production and disturbance of collagen synthesis and degradation, subsequently forming fibrosis of lung and skin lesion. In addition, our findings suggested that silica may influence and alter circulating lymphocytes to disturb autoimmune tolerance [37, 38].

On the other hand, a consideration of the complications of asbestos exposure indicates that the development of cancers is the most important aspect [1–5]. As silica can affect immunocompetent cells, asbestos may possess a similar influence on various immune cells, and the results should be the reduction of tumor immunity. As mentioned above, in addition to lung cancer and mesothelioma, there may be a relatively high prevalence of other cancers among asbestos-inhaled patients [6, 7].

We have reported that the natural killer (NK) cell line and freshly isolated NK cells derived from healthy donors (HDs) exposed to asbestos (chrysotile) for a long period (more than half a year in vivo for the cell line and approximately two...
 weeks for the fresh NK cells) showed a reduction of cytotoxicity with decreased expression of their activating receptors, such as NKG2D and 2B4 in the cell line and Nkp46 in the fresh NK cells [39]. The NK cell line exposed to asbestos also showed suppressed signaling such as extracellular signal-regulated kinases (ERK) 1/2 in the mitogen-activated protein kinase (MAPK) cascade from activating receptors and also producing of granzyme and perforin [40, 41]. At present, the effects of chrysotile on CD8+ cytotoxic T lymphocytes (CTL) are also being analyzed, and findings show reduction of differentiation from naïve CTL to effector/memory CTL and proliferation [42].

3. Chronic Inflammation and Development of Mesothelioma

Inhaled asbestos is usually handled by alveolar macrophages. Recently, the role of a NOD-like receptor family, the pyrin domain containing 3 (NLRP3) (NACHT, LRR, and PYD domains-containing protein 3; Nalp3) inflammasome, has received attention regarding the handling of these foreign bodies as well as various crystalline substances such as uric acid and cholesterol crystalline causing atherosclerosis [43–47]. The following cellular and molecular events then occur. (1) Capture of silica/asbestos by macrophages and entrapment within lysomes. (2) Activation of NLRP3 inflammasome to cleave procaspase 1 to an active form. (3) Cleavage of prointerleukin (IL)-1β to an active form for release to form fibrotic nodules. (4) Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the macrophages. (5) Induction of cellular and tissue damage due to the production of ROS and RNS. (6) Apoptosis of the alveolar macrophages. (7) Production of various cytokines/chemokines such as IL-1β tumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1/2, monocyte-chemoattractant protein-1, and IL-8 to cause chronic inflammation and proliferation of collagenic fibers. (8) Release of silica particles and asbestos fibers from alveolar macrophages and the repetition of similar cellular reactions described above by newly recognized nearby macrophages. (9) Transfer of silica particles and (partially cleaved) asbestos fibers to regional lymph nodes. (10) As these cellular and molecular reactions are continuously repeated, pulmonary fibrosis will appear gradually and progressively [48, 49].

As a result of these cellular and molecular events, cleaved asbestos fibers will accumulate in regional lymphonodes, the distal end of the alveolus, and the pleural cavity, particularly at the opening of lymphatic vessels (Figure 1) [1–5]. Circulating and local immunocompetent cells may encounter asbestos fibers repeatedly, recurrently, and continuously.

Asbestos, particularly amphibole in the form of crocidolite and amosite, includes iron and is considered responsible for the production of reactive oxygen and nitrogen species (ROS and RNS) that may cause DNA damage to nearby cells and induce the development of cancers [50–52]. In addition, these ROS and RNS may develop local chronic inflammation. It has been thought that chronic inflammation contributes to a substantial part of environmental carcinogenesis [53–55]. Various infectious diseases and physical, chemical, and immunological factors participate in inflammation-related carcinogenesis [50–55]. For example, hepatocellular carcinoma, cervical cancer of uterine, and bladder cancer are known to be caused by infection of hepatitis C virus, human papilloma virus (HPV), and Schistosoma haematobium, respectively. In addition, Helicobacter pylori infection causes gastric cancer, extranodal marginal zone lymphoma of mucosa-associated lymphoid tissue type (MALT lymphoma), and diffuse large B-cell lymphoma. Regarding incidences of these diseases, WHO reported about Hepatitis C virus [56]. As many as 2 to 4 million persons may be chronically infected in the United States, 5 to 10 million in Europe, and about 12 million in India, and most do not know that they are infected. About 150,000 new cases occur annually in the USA and in western Europe and about 350,000 in Japan. Of these, about 25% are symptomatic, but 60 to 80% may progress to chronic liver disease, and 20% of these develop cirrhosis. About 5%–7% of patients may ultimately die of the consequences of the infection. In addition, HPV causes cervical cancer which is the second most common cancer in women worldwide. In 2008, there were an estimated 529,000 new cases and 274,00 deaths due to cervical cancer [57]. It was reported that 8-nitroguanine was formed at sites of carcinogenesis in animal models and patients with various cancer-prone infectious and inflammatory diseases, caused by parasites, viruses, and asbestos exposure. In asbestos-exposed mice, 8-nitroguanine was formed in bronchial epithelial cells, and it is noteworthy that crocidolite induced significantly more intense 8-nitroguanine formation than chrysotile, findings that are inconsistent with their respective carcinogenic potentials [58].

From the above-mentioned basic research (2) concerning “investigation of asbestos-induced carcinogenesis using an animal model” in the “Comprehensive Approach on Asbestos-Related Diseases” project, the importance of iron is supposed even in the development of mesothelioma caused by iron-free chrysotile because of its easy binding to hemoglobin and the induced hemolysis [59–61]. Immunocompetent cells may show some alteration as characteristics of chronic inflammation and features involving reduced tumor immunity.

From this viewpoint, even though we do not observe cellular and molecular changes for immunocompetent cells, we reported interesting findings regarding the reduction of tumor immunity in T cells. We then introduce the findings concerning reduction of CXC chemokine receptor (CXCR) 3 and interferon (IFN)-γ with the activated potential expression of IL-6.

4. Chemokine Receptor CXCR3 Expression and Its Relation To Interferon (IFN)-γ and IL-6

Similar to analyses of NK cells, we adopted a human T-cell line, MT-2, as the chronic and continuous exposure model of T cells. Although the altered features of continuously exposed (to chrysotile) sublines (we established six sublines
independently exposed to chrysotile) of MT-2 were reported previously [62–64], one of the interesting molecular changes regarding tumor immunity is the reduction of CXCR3 expression and IFN-γ production [65–68]. CXCR3 is thought to be important for inflammation, since CXCR3 is known as the receptor for CXCL9, 10, and 11 which induce inflammation. In addition, CXCR3 expressing T cells in the tumor-localized region recruit IFN-γ-producing cells to kill the tumor cells.

Results using the MT-2 cell line model, as shown in the left upper panel of Figure 2, indicated that continuously exposed sublines of MT-2 showed reduced CXCR3 expression on their surface and mRNA expression levels, with reduced production and expression of IFN-γ. Production of the Th1 type CXCR3 ligand CXCL10/IP10 was also significantly reduced in sublines compared with the original line. In addition, another Th1-type chemokine, CCL4/MIP-1β mRNA, was also expressed at low levels in all six sublines compared with the MT-2 original line. These results indicated that continuous exposure of MT-2 original cells to asbestos altered the expression of Th1-related chemokines (CXCL10/IP10 and CCL4/MIP-1β) and chemokine receptors (CXCR3) [69, 70].

Similar to analyses regarding the effects of asbestos on NK cells, we then tried to determine whether freshly isolated human peripheral CD4+ T cells show a similar alteration ex vivo when proliferation is maintained by IL-2-containing medium in the presence of chrysotile as shown in the left lower panel of Figure 2 [69, 70]. After several weeks of coculture supplemented with IL-2 in the presence or absence of chrysotile, cell surface CXCR3 expression decreased in a dose-dependent manner. Thus, we examined cell surface expression of CXCR3 and CCR5 in CD4+ T cells derived from six healthy donors, since both receptors are preferentially expressed in Th1/effector or T cells. The expression of CXCR3 was significantly reduced following exposure to 10 μg/mL of chrysotile for 28 days, although this difference seemed to depend on one case in which the expression decreased remarkably [69, 70]. Even if the culture conditions for the CD4+ T cells was limited to a period of around four weeks, four of the six HDs showed a decrease of CXCR3 expression to various degrees, and it might be concluded that asbestos exposure potentiates reduction of CXCR3 expression in CD4+ T cells. These results indicated that CXCR3 expression might be specifically reduced by asbestos exposure. In addition, these experiments revealed decreased IFN-γ expression and production when CD4+ T cells from HDs were cultured with chrysotile for 28 days [69, 70].

Finally, analyses of changes in surface CXCR3 expression on freshly isolated CD4+ T cells from asbestos-exposed patients such as those with pleural plaque (PP) or malignant mesothelioma (MM) were compared with those from HDs. In addition, IFN-γ and IL-6 expression of CD4+ T cells from these patients and HDs was measured with stimulation using anti-CD3/CD28 antibodies with IL-2 [69, 70]. As summarized in the right panel of Figure 2, CXCR3 expression was reduced in CD4+ T cells from asbestos-exposed patients. A comparison of PP and MM patients showed that the expression level of CXCR3 on CD4+ T cells from MM was decreased, although the difference was not statistically significant. Moreover, although IFN-γ expression was only reduced in stimulated CD4+ T cells from MM patients and not in those with PP, IL-6 expression was gradually enhanced in HDs and to a lesser extent in PP, followed by MM. As we reported previously, the plasma level of IL-6 was significantly higher in MM compared to HDs, PP, and silicosis [71].
Although this may depend on the tumor-producing IL-6 [72–74], our findings indicated that part of the increased IL-6 may be produced by T cells with altered potentials to express cytokines due to continuous exposure to asbestos. Furthermore, IL-6 is an interleukin that acts as a proinflammatory and anti-inflammatory cytokine. It is secreted by T cells and macrophages to stimulate an immune response, for example, during infection and after trauma, especially in the case of burns or other tissue damage leading to inflammation [75–78]. For these reasons, it can be assumed that immunocompetent cells possess cellular characteristics of chronic inflammatory alterations during continuous exposure to asbestos, and they then proceed to result in the reduction of tumor immunity as shown in Figure 3. In addition, the supposed difference of immunological effects between silica and asbestos is shown in Figure 4. However, although there is insufficient evidence for all of these sequential modifications of immunocompetent cells,
an investigation of their long-term alteration may lead to the development of preventive tools for asbestos-induced malignancies. For example, it may be possible to find some physiologically active substances in the plants or microorganisms to modify or recover the altered function of immunocompetent cells to reconstitute the tumor immunity in asbestos-exposed people, and discriminate iron from bodies exposed to asbesots.

5. Conclusion

As shown in Figure 3, the carcinogenic activity of asbestos encompasses the following phenomena: (1) DNA damage caused by ROS/RNS production due to the iron present in asbestos fibers, (2) chromosome tangling to result in DNA damage due to the physical features of asbestos fibers, and (3) adsorption of various carcinogens around the asbestos fibers [59–61]. In addition, the molecular events regarding carcinogenesis found in mesothelioma cells include (1) homogenous deletion of p16^{INK4a}/p19^{ARF} found in more than 90% of cases, (2) inactivation of neurofibromatosis 2 (NF2)/Merlin found in approximately half of the cases, (3) inactivation of the serine/threonine-protein kinase (LATS2) gene in approximately one-third of mesothelioma cell lines and representing candidate for a novel tumor suppressor in MM, and (4) transcription factor, Yes-associated protein (YAP) involved in the NF2/Merlin-hippo signaling pathway and constitutively dephosphorylated by LATS, and usually acting as an oncogene to bind with the TEAD transcription factor to enhance the cell cycle and resistance to apoptosis [79–81].

Asbestos fibers and the cellular and molecular characteristics of mesothelioma cells may lead to the gradual alteration of immunocompetent cells and subsequent development of chronic inflammation and later reduction of tumor immunity. Investigation of the progression of modification in immunocompetent cells caused by exposure to asbestos may lead to the development of novel methods for the prevention of mesothelioma and other asbestos-related cancers.

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Role of Nitrative and Oxidative DNA Damage in Inflammation-Related Carcinogenesis

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1. Introduction

Chronic inflammation induced by biological, chemical, and physical factors has been found to be associated with the increased risk of cancer in various organs [1–3]. Inflammation activates a variety of inflammatory cells, which trigger oxidant-generating enzymes such as inducible nitric oxide synthase (iNOS), NADPH oxidase, and myeloperoxidase to produce high concentrations of free radicals including reactive nitrogen species (RNS) and reactive oxygen species (ROS) [1]. Overproduction of RNS and ROS can change the balance of oxidants and antioxidants and cause nitrative and oxidative stress which contributes to the damage of biomolecules such as DNA, RNA, lipid, and proteins, leading to an increase in mutations, genomic instability, epigenetic changes, and protein dysfunction and play roles in the multistage carcinogenic process.

ROS generate 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG, also known as 8-hydroxydG (8-OHdG)), a marker of oxidative DNA damage [4, 5]. 8-OxodG, a potentially mutagenic DNA lesion, leading to the transversion of G : C to T : A (G → T transversion) [6], has been implicated in cancers triggered by infections [7]. The generation of ROS is not confined to inflammatory processes. Carcinogenic chemicals and their metabolites as well as electron transport chains in mitochondria are able to generate ROS. On the other hand, nitric oxide (NO), a primary initiator of RNS, is generated specifically during inflammation via iNOS in inflammatory and epithelial cells [5, 8]. Overproduction of NO participates in the generation of peroxynitrite (ONOO−), which can lead to the formation of 8-nitroguanine, an indicator of nitrative DNA damage [9, 10]. 8-Nitroguanine undergoes spontaneous depurination in DNA, resulting in the formation of an apurinic site [11]. Incorporated adenine can form a pair with apurinic sites during DNA replication, leading to the G → T transversion [12] (Figure 1). Moreover, apurinic sites might represent major damage that requires error-prone DNA polymerase ζ for efficient trans-lesion DNA synthesis. It was reported that DNA polymerase ζ can efficiently bypass abasic sites by extending from nucleotides
Figure 1: Proposed mechanism of point mutation induced by 8-nitroguanine and 8-oxodG through induction of the G:C → T:A transversion.

Table 1: Nitrative and oxidative DNA damage in inflammation-induced carcinogenesis.

<table>
<thead>
<tr>
<th>Etiologic agent/pathologic condition</th>
<th>IARC classification</th>
<th>Cancer site</th>
<th>Associated neoplasm</th>
<th>Detection of DNA lesions [reference no.]</th>
</tr>
</thead>
<tbody>
<tr>
<td>(I) Infection agent</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Viruses</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV b</td>
<td>1</td>
<td>Cervix and other site</td>
<td>Cervical carcinoma</td>
<td>IHC [38]</td>
</tr>
<tr>
<td>High-risk types</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-risk types</td>
<td>2A</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HCV, HBV b</td>
<td>1</td>
<td>Liver</td>
<td>Hepatocellular carcinoma</td>
<td>IHC [56–59]</td>
</tr>
<tr>
<td>EBV b</td>
<td>1</td>
<td>Nasopharynx</td>
<td>Nasopharyngeal carcinoma</td>
<td>IHC [38, 49, 50], ELISA [49]</td>
</tr>
<tr>
<td>Bacterium</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Helicobacter pylori</td>
<td>1</td>
<td>Stomach</td>
<td>Gastric cancer</td>
<td>IHC [36]</td>
</tr>
<tr>
<td>Parasites</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opisthorchis viverrini</td>
<td>1</td>
<td>Intra- and extrahepatic bile duct</td>
<td>Cholangiocarcinoma</td>
<td>IHC [17, 22–26], HPLC-ECD [23, 27]</td>
</tr>
<tr>
<td>Schistosoma haematobium</td>
<td>1</td>
<td>Bladder</td>
<td>Bladder cancer</td>
<td>IHC [60]</td>
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<tr>
<td>(II) Inflammatory disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asbestos fiber</td>
<td>1</td>
<td>Lung</td>
<td>Mesothelioma, lung carcinoma</td>
<td>IHC [61]</td>
</tr>
<tr>
<td>Reflux oesophagitis Barrett’s oesophagitis</td>
<td>Oesophagus</td>
<td></td>
<td>Oesophageal carcinoma</td>
<td>IHC (In prep.)</td>
</tr>
<tr>
<td>Lichen planus</td>
<td>Oral</td>
<td>Oral squamous cell carcinoma</td>
<td>IHC [62]</td>
<td></td>
</tr>
<tr>
<td>Inflammatory bowel disease</td>
<td>Colon</td>
<td>Colorectal carcinoma</td>
<td>IHC [63]</td>
<td></td>
</tr>
<tr>
<td>Crohn’s disease</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chronic ulcerative colitis</td>
<td>Soft tissue</td>
<td>Malignant fibrous histiocyto ma</td>
<td>IHC (this paper)</td>
<td></td>
</tr>
</tbody>
</table>

This table was adapted and modified from the IARC [2] and Coussens and Werb [1].
HPV: human papilloma virus; HBV: hepatitis B virus; HCV: hepatitis C virus; EBV: Epstein-Barr virus.
DNA lesions: IHC, 8-nitroguanine and 8-oxodG detected by immunohistochemistry; HPLC-ECD: 8-oxodG detected by HPLC-ECD; ELISA: serum 8-oxodG detected by ELISA.
inserted opposite the lesion by other DNA polymerases [13]. Wu et al. suggested that cells deficient in subunits of DNA polymerase ζ were hypersensitive to nitrative stress, and trans-lesion DNA synthesis mediated by this polymerase contributes to extensive point mutations [14]. Additionally, DNA polymerases η and κ were also found to be involved in the incorporation of adenine opposite 8-nitroguanine during DNA synthesis in a cell-free system associated with trans-lesion DNA synthesis leading to the G → T transversion [15]. Therefore, 8-nitroguanine is a potential mutagenic DNA lesion involved in inflammation-mediated carcinogenesis. Relevantly, systematic and comprehensive genome-scale approaches by using the immunoprecipitation-based technique combined with high-density microarrays may be useful to investigate roles of DNA lesions in carcinogenesis [16].

We focus on the roles of nitrative and oxidative DNA damage in infection- and inflammation-related carcinogenesis. We produced a specific anti-8-nitroguanine antibody [17] and examined the localization of DNA lesions by immunohistochemical analysis in animal models and clinical samples (Table 1). Here, we review the effects of RNS-/ROS-mediated DNA damage on genomic instability and epigenetic change in relation to carcinogenesis.

2. DNA Damage in Infection-Related Carcinogenesis

2.1. Liver Fluke Infection and Cholangiocarcinoma. Liver fluke infections of *Opisthorchis viverrini* (*O. viverrini*) are a risk factor for cholangiocarcinoma in Southeast Asia [18]. *O. viverrini* infections are endemic in Khon Kaen province, northeastern Thailand, and Khon Kaen has the highest incidence of cholangiocarcinoma in the world [19]. *O. viverrini* infections induce inflammation in both animal models [20] and humans [21]. Our previous studies showed that 8-oxodG and 8-nitroguanine levels were increased in *O. viverrini*-infected hamsters compared with uninfected control groups [17, 22–24]. In addition, DNA damage was significantly increased in reinfected hamsters compared with animals infected just once [23]. Notably, repeated infection increased iNOS expression and 8-nitroguanine production in the epithelium of bile ducts even after a decrease in inflammatory cells. To elucidate the mechanism involved, we examined the expression of iNOS, NF-κB, and Toll-like receptor (TLR) 2 in mouse macrophage cell lines treated with *O. viverrini* crude antigens [25], suggesting that *O. viverrini* infection induced TLR2 activation with NF-κB-dependent transcription and iNOS expression. Treatment with an antiparasitic drug (praziquantel) significantly improved the DNA lesions [22]. These findings in hamsters were confirmed by the observation that 8-oxodG and 8-nitroguanine accumulated more in cancerous areas than in intrahepatic areas adjacent to tumors in surgical specimens [26]. Furthermore, an epidemiological study of *O. viverrini*-infected subjects and cholangiocarcinoma patients demonstrated that urinary 8-oxodG levels were significantly higher in cholangiocarcinoma patients than in *O. viverrini*-infected patients and healthy subjects and higher in *O. viverrini*-infected subjects than in healthy subjects [27]. The urinary 8-oxodG levels in *O. viverrini*-infected patients significantly decreased two months after praziquantel treatment and were comparable to levels in healthy subjects one year after treatment [27]. These results indicate that *O. viverrini* causes chronic and recurrent inflammation followed by the accumulation of oxidative and nitrative DNA lesions, which may participate in the development of cholangiocarcinomas.

2.2. *H. pylori* and Gastric Cancer. *Helicobacter pylori* is the main cause of chronic gastritis and a potential risk factor for gastric carcinoma [28]. The molecular mechanisms behind *H. pylori*-induced production of ROS/RNS were wide-ranging from activated neutrophils to *H. pylori* itself, as nicely reviewed by Handa et al. [29]. *H. pylori* infections promote the secretion of various inflammatory cytokines, contributing directly to the pronounced inflammatory response. Lipopolysaccharide, a component of Gram-negative bacteria such as *H. pylori*, is a TLR4 ligand that induces inflammatory responses via NF-κB expression [30]. NF-κB, which is involved in the regulation of iNOS, had been reported to function as a tumor promoter in inflammation-associated cancer [31, 32]. In patients with *H. pylori*-induced gastritis or gastric ulcers, iNOS is expressed in the infiltrating inflammatory cells [33]. The expression of iNOS mRNA and protein was significantly increased in the epithelial cells of *H. pylori*-positive gastritis patients compared to *H. pylori*-negative patients [34]. Recently, it was also found that *H. pylori* in a Korean isolate induced the expression of iNOS via AP-1 activation [35]. Our previous study [36] demonstrated that levels of 8-nitroguanine and 8-oxodG in gastric gland epithelium were significantly higher in gastritis patients with *H. pylori* infections than in those without infections. A significant accumulation of proliferating cell nuclear antigen (PCNA) was observed in gastric gland epithelial cells in patients infected with *H. pylori* in comparison to those not infected. Interestingly, the accumulation of PCNA was closely correlated with the formation of 8-nitroguanine and 8-oxodG. Collectively, the host response to *H. pylori* mediated NF-κB expression, resulting in iNOS expression accompanied by 8-nitroguanine and 8-oxodG production in the gastric epithelium. 8-Nitroguanine could be not only a promising biomarker for inflammation but also a useful indicator of the risk of developing gastric cancer in response to chronic *H. pylori* infection.

2.3. HPV and Cervical Carcinoma. Cervical cancer is the second most common cancer among women worldwide and the most common cancer among women in many developing countries [37]. Inflammation is proposed to play an integral role in the development of human papilloma virus (HPV)-induced cervical cancer [1]. Our previous study [38] examined the formation of 8-nitroguanine and 8-oxodG in cells of cervical intraepithelial neoplasia (CIN, grades 1–3) and condyloma acuminatum samples and compared it with the expression of the cyclin-dependent kinase inhibitor p16, considered a biomarker for cervical neoplasia [39–42]. Double immunofluorescence labeling revealed that 8-nitroguanine and 8-oxodG immunoreactivities correlated significantly
with CIN grade. There were no statistically significant differences in p16 expression between CIN and condyloma acuminatum samples. These results suggest that high-risk HPV types promote iNOS-dependent DNA damage, which leads to dysplastic changes and carcinogenesis. Therefore, 8-nitroguanine is a more suitable and promising biomarker for evaluating the risk of inflammation-mediated cervical carcinogenesis than p16.

2.4. EBV and Nasopharyngeal Carcinoma. Nasopharyngeal carcinoma (NPC) is strongly associated with Epstein-Barr virus (EBV) infections [43]. Various transcription factors are known to participate in iNOS expression including signal transducers and activators of transcription (STATs), such as STAT1α and STAT3 [44, 45]. Epidermal growth factor receptor (EGFR) physically interacts with STAT3 in the nucleus, leading to transcriptional activation of iNOS [44]. STAT3 is repeatedly activated through phosphorylation via the expression of latent membrane protein 1 (LMP1) as well as EGFR [46, 47], and interleukin-6 (IL-6) is required for LMP1-mediated STAT3 activation [46]. In addition, LMP1-mediated iNOS expression was reported in EBV-infected epithelium cell lines, which play a role in colonization independent of anchorage and tumorigenicity in nude mice [48]. Using biopsy and surgical specimens of nasopharyngeal tissues from NPC patients in southern China, we performed double immunofluorescent staining to examine the formation of 8-nitroguanine and 8-oxodG [49, 50]. Intensive immunoreactivity to iNOS was detected in the cytoplasm of 8-nitroguanine-positive cancer cells. DNA lesions and iNOS expression were also observed in epithelial cells of EBV-positive patients with chronic nasopharyngitis but weaker than those in NPC patients. No or few DNA lesions were observed in EBV-negative subjects. EGFR and phosphorylated STAT3 were strongly expressed in cancer cells of NPC patients, suggesting that the STAT3-dependent mechanism is important to the carcinogenesis [50]. IL-6 was expressed mainly in inflammatory cells of nasopharyngeal tissues of EBV-infected patients. We also found that serum levels of 8-oxodG were significantly higher in NPC patients than control subjects [49]. Collectively, these findings indicate that the nuclear accumulation of EGFR and activation of STAT3 by IL-6 play a key role in iNOS expression and resultant DNA damage, leading to EBV-related NPC.

2.5. HCV and Hepatocellular Carcinoma. Hepatitis C virus (HCV) is a major cause of chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma throughout the world [51]. Hepatocellular carcinoma arises through genetic alterations in hepatocytes during a chronic HCV infection [52–55]. We investigated the extent of nucleic acid damage in HCV-infected individuals and its change after interferon treatment [56]. Immunoreactivities of 8-nitroguanine and 8-oxodG were strongly detected in the liver of patients with chronic hepatitis C, but not control subjects. 8-Nitroguanine was found to be accumulated in hepatocytes particularly in the perportal area. In the sustained virological responder group after interferon therapy, the accumulation of 8-nitroguanine and 8-oxodG was markedly decreased in the liver. We observed a strong correlation between hepatic 8-oxodG staining and serum ferritin levels, suggesting the iron content to be a strong mediator of oxidative stress and iron reduction to reduce the incidence of hepatocellular carcinoma in patients with chronic hepatitis C [57, 58]. We also demonstrated that oxidative DNA damage widely occurred in the livers of patients with chronic viral hepatitis especially chronic hepatitis C, and the iron load and 8-oxodG-positive hepatocytic count was significantly higher in HCV-infected than in HBV-infected livers [59]. It is plausible that ROS production during chronic HCV infection is the result of high iron levels in hepatic tissues, which lead to progressive liver inflammation and an increased risk of developing liver cancer. These findings indicate that 8-nitroguanine and 8-oxodG are useful as biomarkers for evaluating the severity of HCV-induced chronic inflammation leading to hepatocellular carcinoma and the efficacy of chronic hepatitis C treatment.

3. DNA Damage in Inflammation-Related Carcinogenesis

3.1. Asbestos and Lung Carcinoma. Excessive and persistent production of ROS/RNS by inflammatory cells is considered as a hallmark of the secondary genotoxicity of nonfibrous and fibrous particles including asbestos [66]. Asbestos is a carcinogen (IARC Group1) causing lung cancer and malignant mesothelioma of the pleura and peritoneum [67]. Among the different types of asbestos, crocidolite (blue asbestos) and amosite (brown asbestos) are more potent carcinogens than chrysotile (white asbestos) [67]. Inflammation is a hallmark of the response to exposure to asbestos in both animal and human models [68, 69]. NO and nitrative stress were reported to be involved in the asbestos-derived inflammatory response via myeloperoxidase, a major constituent of neutrophils which generates hypochlorous acid and RNS [70–73]. Myeloperoxidase plays a significant role in asbestos-induced carcinogenesis [74]. However, the precise mechanisms of nitrative DNA damage remain to be clarified. We performed an immunohistochemical analysis to examine the formation of 8-nitroguanine and the expression of iNOS and its transcription factor (NF-κB) in the lungs of mice intratracheally administered asbestos fibers, including crocidolite and chrysotile [61]. 8-Nitroguanine was significantly detected in bronchial epithelial cells of asbestos-exposed groups compared with the untreated group. Interestingly, the immunoreactivities of 8-nitroguanine, iNOS, and NF-κB were significantly higher in the crocidolite-exposed group than in the chrysotile-exposed group. Therefore, the formation of nitrative DNA damage could be one of the mechanisms responsible for the difference in carcinogenic potential between crocidolite and chrysotile.

3.2. Inflammatory Bowel Disease and Colon Cancer. Ulcerative colitis and Crohn's disease, which are referred to as inflammatory bowel diseases (IBDs), are well known as chronic inflammatory diseases in the lower bowel. Epidemiological studies have shown that the incidence of colorectal cancer in IBD patients is greater than the expected incidence
in the general population [75]. We hypothesized that an imbalance of helper and regulatory T-cell functions plays a key role in the pathogenesis of IBD. Therefore, we prepared a mouse model of IBD with an imbalance of Th1 and Th2 and, using double immunofluorescence labeling, revealed that both 8-nitroguanine and 8-oxodG were mainly formed in epithelial cells [63]. iNOS, PCNA, and p53 proteins were also expressed in colon epithelium. We observed by using clinical samples that 8-nitroguanine and 8-oxodG were formed in colon epithelium of patients with ulcerative colitis in the active stage (Figure 2). Of relevance, several studies have shown that iNOS is expressed in epithelial cells in colitis patients [76–78]. In noncancerous colon tissues from patients with ulcerative colitis, iNOS protein levels were positively correlated with p53 serine 15 phosphorylation levels [76]. These results suggest that nitrative DNA damage, as well as oxidative DNA damage, participates in colon carcinogenesis in patients with IBD.

3.3. Oral Lichen Planus and Oral Cancer. Oral lichen planus (OLP) is a chronic inflammatory mucosal disease [79] and a risk factor for oral squamous cell carcinoma (OSCC) [80]. Oral leukoplakia is a precancerous lesion characterized by white plaques and hyperkeratosis [81, 82]. We demonstrated that 8-nitroguanine and 8-oxodG accumulated in oral epithelium of biopsy specimens from patients with OLP, leukoplakia, and OSCC, whereas no immunoreactivity was observed in normal oral mucosa [62, 83]. Colocalization of 8-nitroguanine and iNOS was found in oral epithelium of patients with OLP, leukoplakia, and OSCC. Accumulation of p53 was observed in oral epithelium in OLP and leukoplakia patients, and more prominent expression of this protein was observed in OSCC patients. In addition, the immunoreactivity to PCNA was significantly higher in leukoplakia patients than in normal mucosa, suggesting an increase in cell proliferation [83]. Lee et al. also reported that PCNA and p53 were highly expressed in oral tissues in OLP patients [84]. We conclude that inflammation-mediated DNA damage and additional epithelial cell proliferation promote oral carcinogenesis.

3.4. DNA Damage in Malignant Fibrous Histiocytoma. Malignant fibrous histiocytoma (MFH) is one of the most common soft tissue sarcomas [85, 86] and has a poor prognosis [87, 88]. MFH has been proposed to be accompanied by inflammatory responses [89, 90]. However, the mechanism of its inflammation-induced carcinogenesis is still unclear. We investigated DNA lesions and inflammatory-related molecules including iNOS, NF-κB, and COX-2 [64]. Immunohistochemical staining revealed that the formation of 8-nitroguanine and 8-oxodG occurred to a much greater extent in MFH tissue specimens from deceased patients than in live patients. iNOS, NF-κB, and COX-2 were colocalized with 8-nitroguanine in MFH tissues. It is worth noting that a statistical analysis using the Kaplan-Meier method demonstrated strong 8-nitroguanine staining to be associated with a poor prognosis. Furthermore, our study demonstrated significantly higher levels of both 8-nitroguanine and HIF-1α in the tissue specimens of deceased patients than in those of living subjects. Survival curves analyzed by the Kaplan-Meier method differed significantly between the groups with high and low staining of 8-nitroguanine as well as HIF-1α [65]. These results suggest a significant role for the iNOS-dependent formation of 8-nitroguanine via HIF-1α and NF-κB in the progression of inflammation-related cancer. These results indicate that 8-nitroguanine is involved in not only the initiation of carcinogenesis but also its progression and prognosis in cases of MFH.

4. DNA Damage in relation to Genomic Instability

Genomic instability is a defining characteristic of most carcinogenesis through the accumulation of mutations in several tumor suppressor genes, oncogenes, and genes that are involved in maintaining genomic stability [91]. Events resulting in chromosomal instability, such as amplification and deletions of large segments of DNA, reciprocal and non-reciprocal translocations, aneuploidy, and polyploidy, constitute the large-scale genomic aberrations that characterize the majority of human cancer cells and are thought to accelerate
Figure 3: Colocalization of DDR proteins and DNA lesions. (a) Colocalization of γ-H2AX (green) and 8-nitroguanine (red). (b) Colocalization of phosphorylated ATM (green) and 8-oxodG (red).

carcinogenesis [91, 92]. Degtyareva et al. demonstrated that chronic oxidative DNA damage due to DNA repair defects induced chromosome instability in a Saccharomyces cerevisiae model [92]. Trouiller et al. showed that titanium dioxide, a risk factor for lung cancer, induced oxidative DNA damage, γ-H2AX foci, micronuclei, and DNA deletions, suggesting a link between inflammation-associated DNA damage and genomic instability [93]. The DNA damage response (DDR) is essential for maintaining the integrity of the genome, and a failure of this response results in genomic instability and predisposition to malignancy [94]. Phosphorylated ATM (ataxia telangiectasia mutated) plays a role in DDR to DNA double-stranded breaks. Impaired function of ATM was reported to be involved in DNA damage-induced genomic instability [94, 95]. TNF-α is a proinflammatory cytokine and also acts as an iNOS regulator protein [96, 97]. Natarajan et al. reported that TNF-α induced the formation of 8-oxodG and genomic instability in primary vascular endothelial cells [98]. Yan et al. showed that antioxidants significantly reduced TNF-α-induced genetic damage [99]. Therefore, TNF-α and a dysfunction of ATM could play key roles in the integration between iNOS-mediated DNA damage and genomic instability. Recently, we observed that phosphorylated ATM and γ-H2AX were colocalized with 8-oxodG and 8-nitroguanine in clinical samples of cholangiocarcinoma patients as shown in Figure 3, suggesting that DNA base damage caused double-stranded breaks. DNA lesions were detected only very weakly in normal liver tissues, suggesting that the DNA double-stranded breaks were specific to cancer cells. Our observations also support the idea that highly iNOS-dependent DNA damage causes DNA double-stranded breaks and genomic instability, which play important roles in inflammation-induced carcinogenesis via TNF-α signaling and DDR protein dysfunction.

5. DNA Damage in relation to Epigenetic Change

Diverse cellular functions including the regulation of inflammatory gene expression, DNA repair, and cell proliferation are regulated by epigenetic changes [100]. DNA methylation and histone modifications are the major events involved in epigenetic changes. An important proinflammatory cytokine IL-6 has been reported to control DNA methylation through IL-6-mediated Janus kinase (JAK)/STAT3 pathways [101–105]. We demonstrated that IL-6 modulated iNOS expression via STAT3 and EGFR in EBV-associated nasopharyngeal carcinoma [50]. Accumulating evidence makes it increasingly clear that epigenetic silencing plays an important role in EBV-associated neoplasia [106]. We and our colleagues have found promoter hypermethylation in several candidate genes for tumor suppressor genes [107–110]. Histone modifications play a role in the response to DNA double-stranded breaks through ATM signaling to activate γ-H2AX, resulting in histone ubiquitination and acetylation, and destabilization and conformational changes to nucleosomes lead to DNA repair [111]. RNS cause base lesions, abasic sites, and single-stranded breaks, which may be converted into double-strand
breaks in cells by enzymatic processing, when the damage is in close proximity to or encountered by the replication fork [112]. Collectively, nitrative and oxidative DNA damage may activate epigenetic change via IL-6 signaling and the expression of DDR proteins.

6. Conclusion

We investigated the formation of 8-nitroguanine and 8-oxodG at sites of carcinogenesis in various clinical specimens and animal models in relation to inflammation-related carcinogenesis. We also observed that DNA lesions were formed and significantly increased in *S. haematobium*-induced urinary bladder cancer compared with cancer without such an infection [60]. In addition, Barrett’s esophagus, an inflammation-related disease caused by the reflux of gastric acid, also showed greater DNA damage than normal esophageal tissues (unpublished data). Proposed roles of inflammation-related DNA damage in carcinogenesis on the basis of our findings and studies in the literature [94, 113] are summarized in Figure 4. 8-Nitroguanine and 8-oxodG are formed in various inflammation-related cancers and precancerous regions in an iNOS-dependent manner. TNF-α and IL-6 are proinflammatory cytokines which play roles in the control of iNOS expression via the regulation of NF-κB and STAT3 signaling pathways. 8-Nitroguanine and 8-oxodG are mutagenic lesions resulting in the G → T transversion. This type of mutation has been found to occur in vivo in the *ras* gene and the *p53* tumor suppressor gene in various cancers [114]. Nitrative and oxidative DNA damage induce not only mutations but also genomic instability and epigenetic change via TNF-α and IL-6 activities and DNA double-stranded breaks resulting in the activation of oncogenes and inactivation of tumor suppressor genes, which may lead to inflammation-related carcinogenesis.

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