Oxidative Stress-induced Carcinogenesis and Its Prevention

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What has been Learned from the Studies of Oxidative Stress-induced Carcinogenesis: Proposal of the Concept of Oxygenomics

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Summary Epidemiological studies have demonstrated that oxidative stress associated with a variety of pathological conditions is one of the major causes of carcinogenesis. Reactive oxygen and nitrogen species contribute to genomic alterations, presumably followed by selection of the best-adapted proliferating cells in a given environment. Recent data suggest that there exist common signaling pathways for oxidative stress-associated carcinogenesis. So far, oxidative DNA damage has been assumed to be randomly distributed based on *in vitro* experiments, and localization of oxidative DNA damage in the genome *in vivo* has rarely been studied. However, by the use of novel techniques in combination with constructed genome databases, it was found that the localization of oxidative DNA appears to be not random *in vivo*. We propose to call this rather novel research area “oxygenomics”. Not a few signaling pathways start from the recognition of DNA damage. Possible underlying principles should be elucidated in association with cell type, the function of each genomic location, and its transcriptional activity as well as chromatin status determining epigenetic information. Furthermore, this concept may contribute to the development of novel oxidative stress biomarkers. Thus, oxygenomics is a promising research area.

Key Words: carcinogenesis, chromosomal territory, genome DNA damage, ferric nitrilotriacetate, oxidative stress

Link between Oxidative Stress and Human Cancer

Oxidative stress is associated with a plethora of pathological phenomena, including infection, inflammation, ultra-violet- and γ -irradiation, overload of transition metals and certain chemical agents as well as ischemia-reperfusion

injury [1]. Many epidemiological studies have demonstrated a close association between chronically oxidative conditions and carcinogenesis. For example, chronic tuberculous pleuritis causes a high incidence of malignant lymphoma [2]; asbestosis (asbestos fibers are rich in iron [3]) is often associated with mesothelioma and lung carcinoma [4]; chronic helicobacter pylori infection is associated with a high incidence of gastric cancer [5, 6]; the incidence of colorectal cancer is increased in ulcerative colitis [7, 8]; a high risk for hepatocellular carcinoma is observed in patients with genetic hemochromatosis [9, 10]; severe burn by ultra-

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Table 1. Human and animal carcinogenesis presumably associated with oxidative stress

Etiology	Species	Target organ	Representative evidence
<i>Chemical agents</i>			
Alcohol (ethanol)	H, R	Liver	[65, 66]
Asbestos fiber (iron)	H	Mesothelium, Lung	[3, 4]
Cigarette smoke	H	Lung	[67, 68]
Metals (transition metals except arsenic)			
Arsenic	H	Skin	[69]
		Liver, Lung, Bladder, Kidney	[70, 71]
Cadmium	H, R	Lung, Prostate, Liver, Kidney, Stomach	[72–74]
Chromium	H	Lung	[73–75]
Copper	R	Kidney	[76]
Iron	H	Colon, Liver	[10, 77, 78]
	R, M	Soft tissue, Kidney	[79–81]
<i>Inflammation</i>			
Autoimmune diseases			
Crohn's disease	H	Colon	[82, 83]
Ulcerative colitis	H, M	Colon	[7, 8]
Infection			
Helicobacter pylori	H, MG	Stomach	[5, 6, 84–86]
Hepatitis C (iron)	H	Liver	[87, 88]
Mycobacterium tuberculosis		Lung (lymphoma)	[2, 89]
<i>Nutritional</i>			
Choline deficiency	R	Liver	[90]
<i>Physical agents</i>			
Burn	H	Skin	[91, 92]
Radiation	H	Bone marrow	[13, 93]
UV irradiation	H, M	Skin	[11, 12, 94]

H, human; M, mouse; MG, Mongolian gerbil; R rat.

violet radiation is a risk factor for skin cancer [11, 12]; and γ -irradiation causes a high incidence of leukemia [13]. Representative observations are summarized in Table 1. At least under these circumstances, and probably in other types of carcinogenesis as well, oxidative stress appears to play a major role.

Cancer is one of the leading causes of death in most well-developed countries. It has been established that multiple stepwise alteration of the original genome information is one of the major mechanisms responsible for carcinogenesis [14]. Excess generation of reactive oxygen and nitrogen species causes DNA strand breaks, cross-links and modifications [15–18], leading to alterations in the genomic information in spite of the robust counteractions by repair enzymes and apoptotic pathways [19]. These changes of genetic information are called mutations, which consist of point mutations, deletions, insertions or chromosomal translocations. These events may cause persistent activation of oncogenes or inactivation of tumor suppressor genes [14].

After three decades of intensive study to identify the mutated genes (cancer genes) that are causally implicated in carcinogenesis, a “census” of human cancer genes was recently performed. This study revealed that mutations in more than 1% of genes (291 cancer genes) contribute to human cancer (~80% dominant traits and ~20% recessive traits). Ninety percent of the cancer genes show somatic mutations in cancer, 20% show germline mutations and 10% show both of them. The most common functional domain encoded by cancer genes is the protein kinase [20].

Significance of Oxidative Stress in Cancer Cell Proliferation

Imatinib mesylate (Gleevec®), a tyrosine kinase inhibitor, has recently achieved great success in treating chronic myelogenous leukemia, in which a chimeric oncogene, *bcr-abl*, is generated *via* chromosomal translocation [21], and also in a type of sarcoma called gastrointestinal stromal

tumor [22, 23]. On the other hand, recombinant humanized anti-HER2/c-ErbB2/Neu antibody (Herceptin®) [24] is now clinically used to antagonize the receptor-type tyrosine kinase in invasive ductal carcinoma of the mammary gland [25]. These are exciting advances in medicine, though the cancer cells may acquire resistance at a later stage [26], and stress the importance of specific signaling pathways each cancer has established.

However, we have to recognize that these specific signaling pathways have evolved by selective processes from thousands of possible mutations to establish a “robust” cellular system [27]. Thus far, how cells acquired these mutations has been largely unknown. The significance of oxidative stress in carcinogenesis has been established in the past decade, and is summarized in Figure 1. It is notable that mutation and persistent activation of new signaling pathways for proliferation are cooperative [28]. Selected mutations of oncogenes generate new signaling pathways for constant proliferation, and increased cellular proliferation in turn enhances the mutation rate. Another suggested mechanism is a “mutator phenotype” in which inactivation of caretaker genes for the genome leads to a higher mutation rate [29]. In a sense, carcinogenesis might be compared to evolution, with the difference that carcinogenesis is fatally impatient with respect to time and is mostly associated with somatic cells. Recently, there is great interest in epigenetic alterations in carcinogenesis in terms of histone modification (acetylation and methylation) and methylation of CpG islands of the promoter region [30]. Although there is still no convincing data published on the association of oxidative stress and epigenetic alteration, we believe that there should be some interactions between them, considering the close association between oxidative stress and carcino-

genesis and the frequent involvement of epigenetic shutting-off mechanisms of tumor suppressor genes in carcinogenesis.

Redox regulation is one of the key mechanisms for adapting to a variety of stresses, including oxidative stress [31, 32]. Recently, it was reported from several independent laboratories, including ours, that an antagonizing protein for thioredoxin (thioredoxin-binding protein-2, TBP-2; also known as vitamin D₃ upregulated protein-1, VDUP-1) [33] is down-regulated in cancers including human adult T-cell leukemia [34, 35], human gastric cancer [36, 37] and iron chelate (ferric nitrilotriacetate)-induced renal cell carcinoma of rats [38]. The mechanism of inactivation is consistently methylation of the promoter region. Furthermore, TBP-2 is expressed at higher levels in nonmetastatic melanomas than metastatic melanomas [39]. Studies of a TBP-2-null mutant mouse [40] provided evidence that loss of TBP-2 results in enhanced sulfhydryl reduction and dysregulated carbohydrate and lipid metabolism, namely, hyperinsulinemia, hypoglycemia, hypertriglyceridemia and increased levels of ketone bodies, at least in the liver and pancreatic β -cells [41]. This was confirmed by producing TBP-2-deficient mice [42]. Loss of TBP-2 appears advantageous to cancer cells because it ultimately results in facilitation of the glycolytic pathway *via* enhancing the thioredoxin activity when we consider the fact that cancer cells are gradually placed in a lower oxygen environment as they form a larger tumor [43].

Oxidative Stress and Genome

Most scientists assumed that free radical reactions show little specificity based on *in vitro* experiments, in contrast

Fig. 1. Significance of oxidative stress in carcinogenesis.

to the extremely selective antigen-antibody interactions. Indeed, the second-order rate constant for the reaction of hydroxyl radical with guanine is $\sim 1.0 \times 10^{10} \text{ M}^{-1}\text{s}^{-1}$ [1]. Thus, one might think that the genome is damaged at random and that there are no specific “target” genes or signaling pathways in oxidative stress-associated carcinogenesis. However, it is time to revisit this assumption. We are recently challenging this hypothesis since ferric nitrilotriacetate (Fe-NTA)-induced renal cancers are rather homogeneous in histology [17, 44]. At the early stage of this rat carcinogenesis model, increased amounts of oxidatively modified DNA bases including 8-oxoguanine [45] and a major lipid peroxidation product, 4-hydroxy-2-nonenal, and its proteins modified by this moiety [46–49] are observed.

In order to clarify whether there is any target tumor suppressor gene, we used a genetic strategy employing microsatellite analysis in F1 hybrid rats [50]. This study revealed that *p15^{INK4B}* (*p15*) and *p16^{INK4A}* (*p16*) tumor suppressor genes are two of the major target genes. This was the first report that showed the presence of a target gene in the free radical-induced carcinogenesis model [51]. The significance of this finding is enormous since *p16* is associated not only with the retinoblastoma protein pathway as a cyclin-dependent kinase 4 and 6 inhibitor, but also with the TP53 pathway *via* *p19^{ARF}* and MDM2 [52]. *p19^{ARF}* is an alternatively spliced transcript from the *p16* tumor suppressor gene [53]. Indeed, iron-mediated oxidative damage appears to attack one of the most critical loci of the genome. We later showed that allelic loss of *p16* occurs as early as 1 week after the start of the animal experiment and is *p16* gene-specific [54]. We believe that these results suggest the presence of fragile sites in the genome. Thus,

the next question is whether carcinogenesis is a process of “random alteration of genetic information followed by selection” or “non-random alteration of genetic information followed by selection.” The final conclusion may change the chemopreventive strategy in particular cases of carcinogenesis.

Oxygenomics

Studying the localization of oxidative nucleic acid damage in comparison with genome information and cellular structure is becoming increasingly important. There are not a few reports published on oxidative DNA damage *in vitro* using purified DNA or cultured cells. Based on these data, it has been claimed that certain specific sequences, including telomeres [55, 56], are especially vulnerable to oxidative damage. However, currently, limited data are available on which part of the genome is susceptible to oxidative damage *in vivo* in individuals. The results obtained *in vitro* have to be confirmed at the tissue and organ levels step by step.

We believe that this is now possible given the completion of genome projects of humans, mice, rats and other species (<http://www.ncbi.nlm.nih.gov/Genomes/>). Studies are now in progress to make libraries of ~ 1 kilobase DNA fragments that contain one or more 8-oxoguanine [57] or acrolein-modified adenine [58] by applying the principle of immunoprecipitation [59] (Fig. 2). One must be aware that nuclear genomic DNA in association with histones is integrated into the chromatin structure in the cell, and that some parts of the chromatin structure are open for transcription. Genome information, the blueprint for a cell, is not

Fig. 2. Oxygenomics as a means to analyze the *in vivo* genomic DNA status in terms of oxidative stress.

continuous information, but is divided into several pieces by the existence of chromosomes, although we cannot observe the chromosomal structure at interphase. Recently, the concept of chromosome territory was established [60, 61]. This concept indicates that genome information corresponding to each chromosome is located at a rather fixed site in the nucleus even at the interphase, namely nuclear central or peripheral. The localization appears to be different among different kinds of cells [62]. It is possible that the genome areas susceptible to oxidative stress may differ depending on the kind of cells and the situation in which the cells are placed. Such differences could help to explain the different signaling pathways each type of cancer has acquired since not a few signaling pathways start from the recognition of DNA damage. We propose to call this novel research area "oxygenomics." Oxygenomics is defined as a research area studying the localization of oxidative DNA damage in the genome in living cells.

As described above, tailored cancer therapy is now being developed. Cancer prevention [63, 64] is not less important than cancer therapy, considering the economic impact of current medical therapeutic costs. In the near future, tailored cancer prevention will become an important intervention when one has a high risk for a certain type of cancer. It will be essential to establish reliable organ-specific biomarkers for oxidative stress as well as for oxidative stress-induced preneoplastic lesions.

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