

CHAPTER II

LITERATURE REVIEW

2.1 Inflammation and cancer

The functional relationship between inflammation and cancer is not new. In 1863, Virchow hypothesized that the origin of cancer was at sites of chronic inflammation, in part based on his hypothesis that some classes of irritants, together with the tissue injury and ensuing inflammation they cause, enhance cell proliferation. Although it is now clear that proliferation of cells alone does not cause cancer, sustained cell proliferation in an environment rich in inflammatory cells, growth factors, activated stroma, and DNA-damage-promoting agents, certainly potentiates and/or promotes neoplastic risk (Coussens and Werb 2002). Epidemiological studies indicate a strong relationship between inflammation and carcinogenesis. For example, individuals with long-standing extensive ulcerative colitis and Crohn's disease have a significant risk of colorectal cancer (Siegel and Sands 2006), chronic hepatitis B and C infections in the liver predispose to hepatocellular carcinoma (Barazani, Hiatt et al. 2007), and *Helicobacter pylori* infection has been established to have a causal relationship to gastric cancer (Peter and Beglinger 2007). Within the lung, chronic inflammatory diseases, such as idiopathic pulmonary fibrosis, systemic sclerosis, certain pneumoconiosis and chronic obstructive pulmonary disease (COPD), have been implicated to lung carcinogenesis (Brody and Spira 2006).

For the lung, experimental studies with rats, as well as molecular epidemiological studies in humans, have provided evidence that the influx of neutrophils into the airways may be an important process linking inflammation with carcinogenesis. Currently it is believed that the genotoxic capacity of neutrophils is a crucial aetiological factor in this carcinogenic response (Knaapen, Gungor et al. 2006). Weitzman and Stossel first demonstrated that



activated polymorphonuclear neutrophils (PMNs) are able to cause both mutations (Weitzman and Stossel 1981) and malignant transformations in vitro (Weitzman, Weitberg et al. 1985). Further studies have supported the important role of PMNs in carcinogenesis by the ability of PMNs to induce DNA single strand breaks (Shacter, Beecham et al. 1988; Knaapen, Schins et al. 2002) and DNA base modification (Dizdaroglu, Olinski et al. 1993; Knaapen, Seiler et al. 1999). Although the mechanisms whereby inflammation may initiate or promote carcinogenesis has not been fully elucidated, production of DNA damaging reactive oxygen species (ROS) by activated inflammatory cells has been proposed to contribute significantly to inflammation-associated carcinogenesis (Ohshima, Tatemichi et al. 2003). Reactive oxygen species are nowadays considered to participate in cancer initiation, promotion and progression. Reactive oxygen species are highly reactive molecules or molecular fragments that are continuously produced in all aerobic organisms, mostly as a consequence of mitochondrial respiratory chain reaction. Besides oxidative phosphorylation, ROS are continuously formed in peroxisomes, the cytochrome P450 system and by inflammatory cells, including neutrophils, eosinophils and macrophages (Karihtala and Soini 2007).

Compared to other organs, the lung represents a unique tissue for oxidant stress because it is directly exposed to higher oxygen concentration. In addition, because of their direct exposure to ambient air, lung cells experience enhanced oxidant stress by environmental irritants and pollutants including oxidants such as cigarette smoke, ozone, and free radical-generating environmental carcinogens (Kinnula and Crapo 2003). Inhaled particles such as crystalline and those present in cigarette smoke and diesel exhaust can stimulate oxidant generation by inflammatory cells, and it is suggested that pulmonary carcinogenicity upon chronic particle exposure involves an influx and subsequent activation of inflammatory phagocytes (Knaapen, Borm et al. 2004).



2.2 Genotoxicity of HOCl

Upon activation, neutrophils generate a vast amount of oxidants. PMN-generated ROS is suggested to be a major factor involved in the mechanism by which neutrophils induce mutations and promote cancer development. Neutrophils contain the enzyme myeloperoxidase (MPO), which is capable of catalyzing the reaction of chloride with hydrogen peroxide to produce large amounts of hypochlorous acid (HOCl) as an end product of the respiratory burst upon neutrophil activation (Knaapen, Gungor et al. 2006). Currently, it is generally accepted that the mutagenic capacity of ROS-derived neutrophils is mediated by H_2O_2 through the formation of highly reactive hydroxyl radical ($\cdot OH$) (Spencer, Jenner et al. 1995). In contrast, until recently HOCl was believed to have no contribution in DNA damage and mutagenesis mediated by activated neutrophils (Shacter, Beecham et al. 1988). However, recently we found that hypoxanthine-guanine phosphoribosyl transferase (HPRT) gene mutation frequency was increased in HOCl-exposed A549 human alveolar epithelial cells (Güngör *et al.*, Unpublished). In line with this observation, Güngör *et al.* showed that neutrophils were potent inhibitors of nucleotide excision repair (NER) in human pulmonary epithelial cells and MPO-catalysed formation of HOCl was thought to be the most likely ROS responsible for these inhibitory effects (Gungor, Godschalk et al. 2007). In fact, HOCl is the major oxidant produced by neutrophils, since MPO consumes up to 70% of neutrophil-derived H_2O_2 to generate HOCl (Hampton, Kettle et al. 1998). Hypochlorous acid plays an important role in bacterial cell killing, but excessive or misplaced generation of HOCl is known to cause damage to tissues. HOCl is capable to react with a number of biological molecules including DNA, proteins, lipids and cholesterol (Hawkins and Davies 2002). In addition, in the presence of superoxide ($O_2^{\cdot -}$) or reduced metal ions (Masuda, Suzuki et al. 2001), HOCl may generate hydroxyl radicals. Shen *et al.* reported that incubation of DNA with either isolated myeloperoxidase (MPO) or eosinophil peroxidase (EPO), together with



plasma levels of halides (Cl⁻ and Br⁻), and a cell-free O₂^{•-}-generating system resulted in oxidative DNA damage (Shen, Wu et al. 2000). However, despite the multitude of cellular and extracellular targets with which HOCl can react, its ability to cause DNA damage in intact cells is still poorly investigated (Spencer, Whiteman et al. 2000). Therefore, our recent findings regarding HOCl-induced mutagenicity, warrant further studies to investigate the oxidative DNA damage effects of HOCl in order to find a possible explanation for the mutagenic effects of HOCl.

2.3 Cellular response to DNA damage

Various mechanisms exist to maintain genetic stability of cells facing DNA damage. The cellular response to DNA damage is complex and most of which link to the cell cycle. The inactivation of cell cycle checkpoint control has emerged as a central cause of genetic instability, ultimately leading to increased susceptibility of cells to consequences of DNA damages. Consequently, these unstable cells may contribute significantly to the origin of cancer (Samuel, Weber et al. 2002).

The *p53* tumor suppressor is the most frequently mutated gene in all of human cancers and is a universal sensor of genotoxic stress. Upon phosphorylation following genotoxic stress, *p53* cellular protein levels accumulate. The protein translocates to the nucleus where a combination of phosphorylated and acetylated *p53* tetramers bind to target gene *p53*-responsive elements to orchestrate the necessary downstream target gene expression to influence cellular growth arrest, apoptosis, or adaptation to DNA damage. The effects on *p53* stability and target gene expression are distinct steps. Control of *p53* stability is governed by its interaction with its negative regulator, MDM2. The *p53*-MDM2 interaction becomes disrupted upon phosphorylation at the amino-terminus of *p53* (Colman, Afshari et al. 2000).

