SCIENTIFIC REVIEWS

Lung Carcinogenesis as a Result of Oxidative Stress and Inflammation

Reactive oxygen species, oxidative stress, pulmonary inflammation and activation of transcription factors play important roles in lung carcer

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Abstract. Carcinogenesis of the respiratory system has been a widespread disease. In 2012 a total of 1.8 million lung cancer new cases were recorded, accounting for around 13% of all new cancer diagnoses. The most important risk factors for respiratory system cancers is tobacco smoking (active and passive). Occupational exposures to carcinogens (asbestos, diesel fumes, silica dust, radon, nickel, chromium, etc) are important causative factors. Also, ambient and indoor air pollution from particulate matter (PM_{10}, $PM_{2.5}$, $<PM_{2.5}$) contribute substantially to initiation and promotion of lung cancer. Human lung tissues are exposed daily to air oxidants generated either endogenously or exogenously, but all aerobic organisms are protected against oxidative damage by evolutionary enzymatic and non-enzymatic antioxidant systems. Respiratory cancers and in particular lung cancer are initiated through the generation of reactive oxygen and nitrogen species (ROS/RNS), leading to oxidative stress and tissue inflammation. Both play a fundametal role in the initiation and progression of cellular and mitochondrial DNA damage, membrane lipid peroxidation and oxidative damage to proteins. Ambient PM has the ability to penetrate the respiratory system and particles are trapped deep inside the lung's alveoli. The physicochemical characteristics of particles (size, transition metal content, speciation, stable free radicals) play an important role in oxidative stress. In turn oxidative stress from chronic exposure initiates the synthesis of mediators of pulmonary inflammation in lung epithelial cells and contributes to the initiation of carcinogenic mechanisms. Pulmonary cancer mechanisms have been linked to a series of biochemical pathways of DNA and lipid membrane oxidative damage, macrophage stimulation, telomere shortening, modulation of gene expression and activation of transcription factors with important role in carcinogenesis. This review presents scientific papers on the role of ROS and oxidative stress in the production of mediators of pulmonary inflammation and mechanisms of carcinogenesis from selected recent studies and reviews (most from high impact scientific publications) on the risks factors for developinmg cancer of the respiratory system.

Introduction

Pulmonary carcinogenesis and in particular lung cancer has been the most worldwide diagnosed type of cancer for several decades, according to the International Agency for Research on Cancer (IARC-WHO, Lyon). The major types of lung cancer include adenocarcinoma, squamous cell carcinoma, small cell and large cell carcinoma.[1]

It is estimated that lung cancer recorded 1.8 million new cases worldwide in 2012 (IARC-The Global Intitiative for Cancer Registry Development, an international partnership) or ~13% of the total cancer incidence), of which 1.2 million cases were in men (66%). From this total, 58% of lung cancer occurred in the less developed regions of the world where smoking is prevalent. Also, lung cancer is the most common cause of death from cancer diseases worldwide, responsible of nearly 20% of the total. The most commonly diagnosed cancers worldwide were lung (1.82 million), breast (1.67 million), and colorectal cancer (1.36 million). Despite the progress in prevention, treatment and anticancer drugs, the most common global causes of cancer deaths were lung cancer (1.6 million deaths), liver cancer (745,000 deaths), and stomach cancer (723,000 deaths). [1,2]

Scientists of IARC and worldwide collaborators, after collecting statistical data from 185 countries (for 36 types of cancers) announced that in 2018 there will be 18.1 million new cancer cases globally (17.0 million excluding nonmelanoma skin cancer) and 9.6 million cancer deaths (9.5 million excluding nonmelanoma skin cancer). In both sexes combined, lung cancer is the most commonly diagnosed cancer (11.6% of the total cases) and the leading cause of cancer death (18.4% of total deaths), closely followed by female breast cancer (11.6%), prostate cancer (7.1%), and colorectal cancer (6.1%). Lung cancer is the most frequent type of cancer and the leading cause of cancer death among males.[3]

All epidemiological studies agree that the most important risk factor for lung cancer is tobacco smoking. It must be emphasized that active smoking is substantially more dangerous, but also passive smoking causes adverse health effects and to a smaller extend lung cancer. Years of research established without doubt that there is a clear association of smoking and various types of respiratory

cancers, but especially lung cancer. Tobacco smoking is involved in synergistic actions with other carcinogenic chemicals and accounts for more than 25% of various types of cancer deaths (not only lung cancer). Global trends in lung cancer are associated with smoking prevalence among adult population. Most developed countries advanced for decades rigorous anti-smoking campaigns promoting smoking cessation (or quitting smoking). Policies making workplaces and public places smoke-free were very positive. These campaings convinced millions of smokers to kick the habit in the last decades. As a consequence, many high-income countries (USA, W. Europe, Scandianvian countries, Australia, Japan, etc) have seen a considerable decrease in smoking prevalence and consequently lung cancer incidence and mortality started to decline. Despite major achievements in worldwide campaings of tobacco control, with current smoking patterns, lung cancer will remain a major cause of death worldwide for several decades.[4-7]

The World Health Organization (WHO) of the United Nations has a target to promote smoking cessation worldwide and reduce smoking by 1/3 by 2030. Studies in developed countries, such as the Million Women Study in the UK, have shown the extraordinary extent to which quitting smoking has positive effect on premature morbidity and mortality. This study was fiunde by the Cancer Research-UK and involved 1.2 million women in the United Kingdom. The results found that the extra risk of dying from smoking (especially lung cancer and other resiratory tyupes of cancer) almost disappears is people stop tobacco smoking before the age of 30, while smoking cessation before the age of 40 avoids more than 90% of premature deaths.[8]

Although smoking is considered major factor of lung cancer, there are also other important contributory factors, such as occupational exposures to carcinogens, ambient air pollution (especially inhalable particulate matter, PM), indoor air pollutants from coal and waste burning in non-ventilated kitchens (in Third World countries), indoor exposure to radon (radioacitve element); ionizing radiation (α-, γ- rays) asbestos fibers (occupational exposure); carcinogenic metals (Cr, Cd, Ni, As, for miners). Also, lung cancer is caused by exposure to some organic chemicals with carcinogenic potential (polychlorinated compounds, dioxins). Industrial occupational exposures, such as in coal gasification, coal-tar pitch, iron

and steel founding, rubber production, diesel fumes and silica dusts are considered dangerous for developing lung cancer after long-term exposure.[9,10]

Reactive oxygen species and oxidative stress

Respiratory diseases are linked to extrinsic causes that promote intitially oxidative stress leading to chronic inflammatory processes after long-term exposure. Many studies showed that smoking is responsible for pulmonary inflammatory processes which in turn increase macrophage recruitment, delayed neutrophil clearance, and initiate oxidative stress. Oxidative stress results from the increased production of free radicals and reactive oxygen species (ROS) in the lung tissues. The pulmonary diseases that are associated with the greatest risk for lung cancer are characterized by abundant and deregulated inflammation. For example, chronic obstructive pulmonary disease and emphysema are characterized by profound abnormalities in inflammatory and fibrotic pathways. Cytokines, growth factors and the developing tumour microenvironment have been found to have deleterious properties that simultaneously pave the way for both epithelial-mesenchymal transition and destruction of specific host cell-mediated immune responses.[11,12]

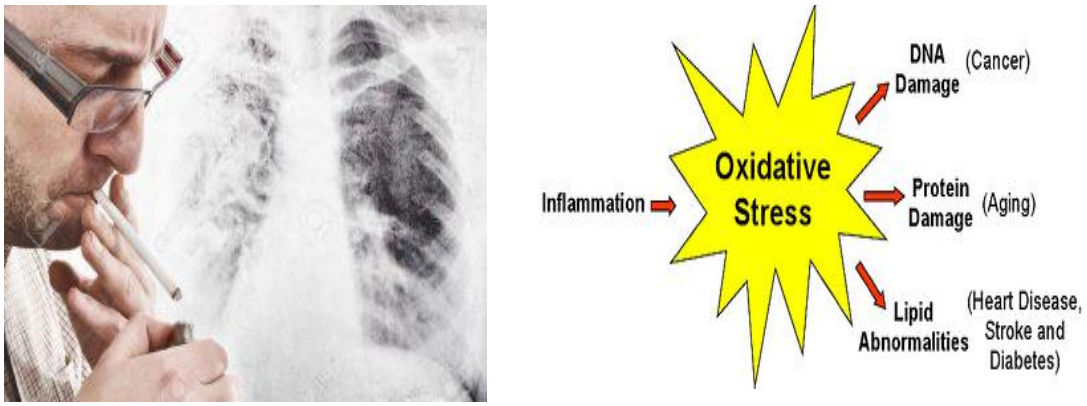

Figure 1. Tobacco smoke contains large number of stable free radicals (tar), oxidants and ROS (semivolatile particles, gases) which can cause oxidative stress to tisssues of the respiratory system, through the oxidative damage to membrane lipids, DNA breaks, damage to enzymes and mitochondrial DNA (mtDNA).

In addition to smoking, respiratory inflammation in humans can be intitated from exposure to various inhalable dusts, particles of heavy metals and mineral fibers (such as asbestos). Also, ambient airborne particulate matter of fine and

superfine size (PM_{10}, $PM_{2.5}$, <PM), ozone (O_3) and diesel fumes have been implicated with lung carcinogenesis. In recent years, ambient air pollution, especially of aerodynamic diameter of 2.5 μm ($PM_{2.5}$) or smaller has gained particular attention as a causative factor in the increased incidence of respiratory diseases, including respiratory cancer. Systematic reviews in the last years provided evidence for the relationship of PM exposure and lung cancer. Also, particulate matter (PM) of ambient air pollution was designated by IARC as a carcinogen in Group 1.[13-18]

Also, these exposure factors can act synergistically with tobacco smoke increasing the risk for epithelial inflammation and subsequently pulmonary diseases including lung cancer. Most research until now indicate that carcinogenic potential of particles, fibers and dusts increases substantially due to the synergistic effects with tobacco smoking carcinogens to generate free radicals and ROS and catalyze redox reactions in human lung epithelial cells, leading to oxidative stress and increased production of mediators of pulmonary inflammation.[19-22]

Size and composition of respirable particles play an important role in the penetration, retention and clearance inside the respiratory system. Fine and superfine particles can penetrate into the respiratory airways and deposited in the respiratory bronchioles and alveoli. The longer a particle is retained the greater the potential to cause harm, such as fibrosis, emphysema or tumour initiation. Particles' durability, solubility and reactivity also are very important. Some particles can be broken down and transported out of the lung by both mucociliary clearance and macrophage transport. But superfine particles or fiberous particles are more hazardous due to their ability to penetrate deep into the lung and resist clearance from the lung interstitium.[23,24]

Inhalable particles from combustion sources contain a number of chemical constituents that can generate ROS by a variety of redox reactions. The most important are transition metals with redox properties (e.g. Fe^{2+}), persistent free carbonaceous radicals, redox-cycling quinones, polycyclic aromatic hydrocarbons (PAHs) and volatile organic compounds (VOCs) which may be metabolically activated to ROS that can react to form bulky adducts or strand breaks on cellular DNA.[25-28]

A number of scientific research projects have linked air pollution particle exposure to oxidation of DNA in cells, tissues, or their metabolites in urine of rodents

and humans. Studies have investigated the effect of diesel exhaust particles (DEP) or ambient air pollutants in terms of oxidized DNA nucleobases.[29,30]

In the last decade a prominent free radical-induced oxidative lesion [In nuclear DNA and mitochondrial DNA (mtDNA)] has been used widely as a predominant biomarker for oxidative stress and carcinogenesis in experimental animals and humans. The 8-oxo-7, 8-dihydro-2-deoxyguanosine (8-oxodG) and methylguanine (N7-MeG) can be measured quantitatively by HPLC and GC-MS in animal and in human biomonitoring studies. These biomarkers are correlated with oxidative and methylated DNA damage by many carcinogenic factors. Thus, it is meaningful to explore the mechanisms of mutagenesis and carcinogenesis associated with oxidative DNA damage by simultaneously measuring those two markers. [31-33]

Additionaly, ozone (O_3) is a strong pulmonary irritant and causes oxidative stress, inflammation and tissue injury. Studies showed that the human bronchiolar epithelium is highly susceptible to injury and oxidative stress induced by acute exposure to ozone, accompanied by altered lung functioning. Epidemiological and clinical studies showed that people exposed to combined air pollutants, such as ozone and cigarette smoke or ozone and ambient particulate matter, have increased pulmonary oxidative stress and inflammation associated with an increase in pulmonary diseases and mortality.[34-36]

Aerobic biological systems and oxidative stress

The appearance of eukaryotic cells, around 2 billion years ago, on Earth has been linked to the increase of the concentration of oxygen (O_2) in the atmosphere and therefore initiation of aerobic metabolism in biological systems. Mitochondria in eukaryotic cells became the powerhouses of the cell with energy-releasing activities of electron transport and proton pumping with the energy conserving process of oxidative phosphorylation. All aerobic biological systems use oxygen as an essential part of their physiological cellular metabolic processes. At moderate concentrations oxygen free radicals, or more generally, reactive oxygen species (ROS) and reactive nitrogen species (RNS), are products of physiological cellular metabolism. In aerobic organisms most of these ROS and RNS are part of the physiology and beneficial to

biological mechanisms. These normally low-concentration compounds that are derived from oxidative metabolism are necessary for certain subcellular events, including signal transduction, enzyme activation, gene expression, etc. But less than 5% of them escape metabolic processes and can be toxic for the cell if their concentration increases.[37-39]

The main sources of endogenous generation of ROS in all cells of aerobic organisms are mitochondria, cytochrome P450 and peroxisome. The majority of ROS are produced in cellular sites by electron transfer reactions through enzymatic and non-enzymatic processes. Under physiological conditions, there is a constant endogenous production of reactive intermediates of radical species of oxygen and nitrogen that interact as regulatory mediators of signaling processes for metabolism, cell cycle, intercellular transduction pathways, cellular redox systems and mechanisms of apoptosis.[40-44]

ROS are diverse and abundant in biological systems. A small proportion of cellular ROS generated endogenously escape the cellular antioxidant defense systems, enzymatic and non-enzymatic (small molecular weight antioxidants). Although most studies showed that the major role in antioxidant defense is fulfilled by antioxidant enzymes, not by antioxidants. Also, exogenous sources, such as tobacco smoke, carcinogenic metals and toxic substances, increase the ROS levels in biological systems. Healthy levels of antioxidant defenses protect aerobic organisms. But any decrease in the cellular antioxidant capacity and increase in ROS and RNS concentrations results in oxidative stress. Cellular ROS sensing and metabolism are tightly regulated by a variety of proteins involved in the redox (reduction/oxidation) mechanism. An imbalance between oxidants and antioxidants in cells of biological systems, in favour of the oxidants, potentially leading to damage, is termed "oxidative stress". Oxidative stress (perturbation of cell redox balance) for extended periods plays a pivotal role in the development of human degenerative diseases and aging. Human lungs are exposed daily to air oxidants generated either endogenously or exogenously. Lung cells are protected against oxidative stress by well-developed enzymatic and nonenzymatic antioxidant systems.[45-47]

But, increased concentrations in internal tissues of free radicals ($R^{\bullet}$) and ROS can cause oxidative damage to all major cellular constituents (membrane lipids,

proteins, enzymes, DNA, RNA, mtDNA), despite the fact that aerobic organisms through evolutionary processes have developed appropriate defenses. Many of the ROS-mediated responses (antioxidant enzymes) actually protect the cells against oxidative stress and re-establish "redox homeostasis". Excessive or sustained increase in ROS production, which if not balanced by antioxidant enzymes and simple antioxidant molecules (e.g. vitamin C) has been implicated in chronic inflammatory conditions (chronic obstructive pulmonary disease or COPD), initiation of malignant neoplasms, diabetes mellitus, atherosclerosis and various neurodegenerative diseases.[48-51]

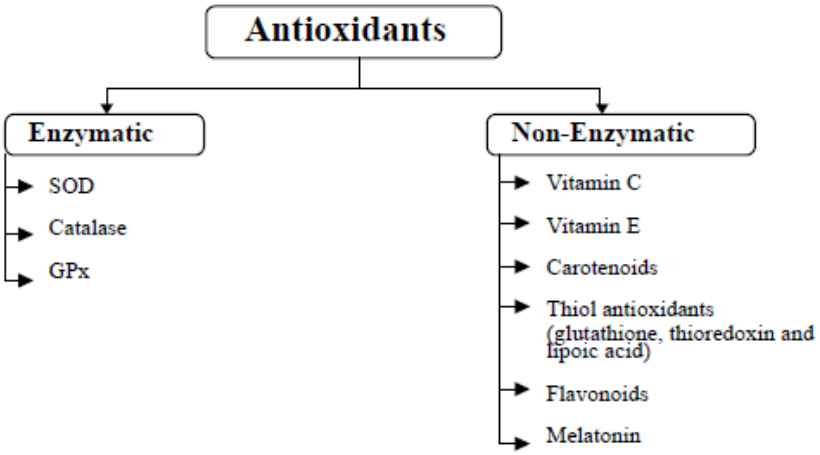

Figure 2. Enzymatic and non-enzymatic antioxidants are capable of preventing (by scavenging or trapping free radicals) the oxidation of biological molecules by ROS and reduce the development of oxidative stress (SOD, superoxide dismutase, GPx, Glutathione peroxidase, is a selenium-containing antioxidant enzyme.

Free radicals and reactive oxidant species can be classified into four groups: Reactive oxygen species (ROS), reactive nitrogen species (RNS), reactive sulfur species (RSS) and reactive chlorine species (RChS). Since oxygen is funtametal molecule in aerobic metabolism, ROS are inevitably the most abundant and highly oxidative. The most important ROS include superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), the highly reactive hydroxyl radical ($HO^{\bullet}$), singlet oxygen ($^1O_2$), ozone (O_3) and others. The most abundant RNS is nitric oxide ($NO^{\bullet}$), which is able to react with certain ROS, including the peroxynitrite anion ($ONOO^-$, interaction of $O_2^{\bullet -}$ and $NO^{\bullet}$). Nitric oxide can be converted into peroxynitrous acid and ultimately into hydroxyl radical and nitrite anion (NO_2^-).[52]

The enzymatic antioxidants in aerobic organisms can intercept, scavenge, and neutralize free radicals and ROS, but also can reactivate intermediates generated in excess under physiological conditions. The most important antioxidant enzymes are: superoxide dismutase (SOD), catalase (CAT), the glutathione redox system (glutathione peroxidase, GPx, and glurtathione-S-transferase). Also, low molecular mass antioxidant compounds are vital for reducing oxidative stress: ascorbic acid (vitamin C), tocopherol (vitamin E), uric acid, bilirubin, glucose, caeruloplasmin, etc, and proteins that bind to metal ions (metallothioneins) blocking free radicals. These antioxidants are part of the evolutionary process of biological aerobic systems.[53]

Exposure to xenobiotics (food, environmental stressors, toxic chemicals, toxins, etc) are potentially damaging events that directly or indirectly challenge redox homeostasis. The capacity to deal with these external challenges is indispensable for life. The balance between oxidants and antioxidants in biological systems is called "*redox homeostasis*", which is a crucial event in living aerobic organisms. Subjecting cells to oxidative stress (pro-oxidative/pro-inflammatory pathways) can result in severe metabolic dysfunction and oxidative damages to proteins, enzymes, carbohydrates, DNA, RNA, mtDNA and membrane lipids.[54-56]

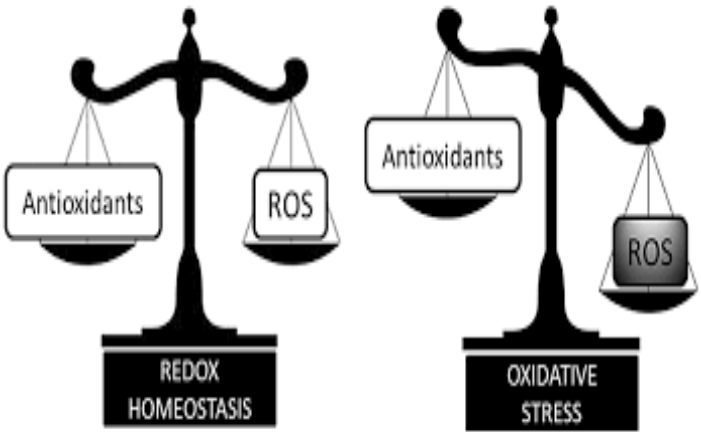

Figure 3. The balance between oxidants and antioxidants in biological systems is called "redox homeostasis", which is a crucial event in living aerobic organisms. Excessive or sustained increase in ROS production, not balanced by antioxidant defences, is leading to oxidative stress.

Inflammatory processes and initiation of carcinogenesis

A correlation between inflammation and cancer was identified in 1863, by Rudolf Ludwig Carl Virchow, who recognized the inflammatory process as one of the

predisposing conditions for tumour development. Although it is now accepted from the majority of scientists that chronic inflammation plays an essential role in tumorigenesis, the underlying molecular mechanisms linking inflammation and cancer remain to be fully explored. Oxidative damage through ROS and other oxidants and subsequent oxidative stress situations are considered to play a pivotal role for promoting several degenerative diseases, cancer and ageing. Oxidative acute and chronic inflammation has been correlated with increased risk for the intitiation and progression of various malignant neoplasms from a great number of clinical and epidemiological studies. The possible mechanisms by which inflammation can contribute to carcinogenesis include genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumour neovascularization, invasion through tumour-associated basement membrane, angiogenesis and metastasis. ROS serve as effector molecules participating in host defense or as chemo-attractants recruiting leukocytes to wounds, thereby influencing the inflammatory reaction in damaged tissues.[57-60]

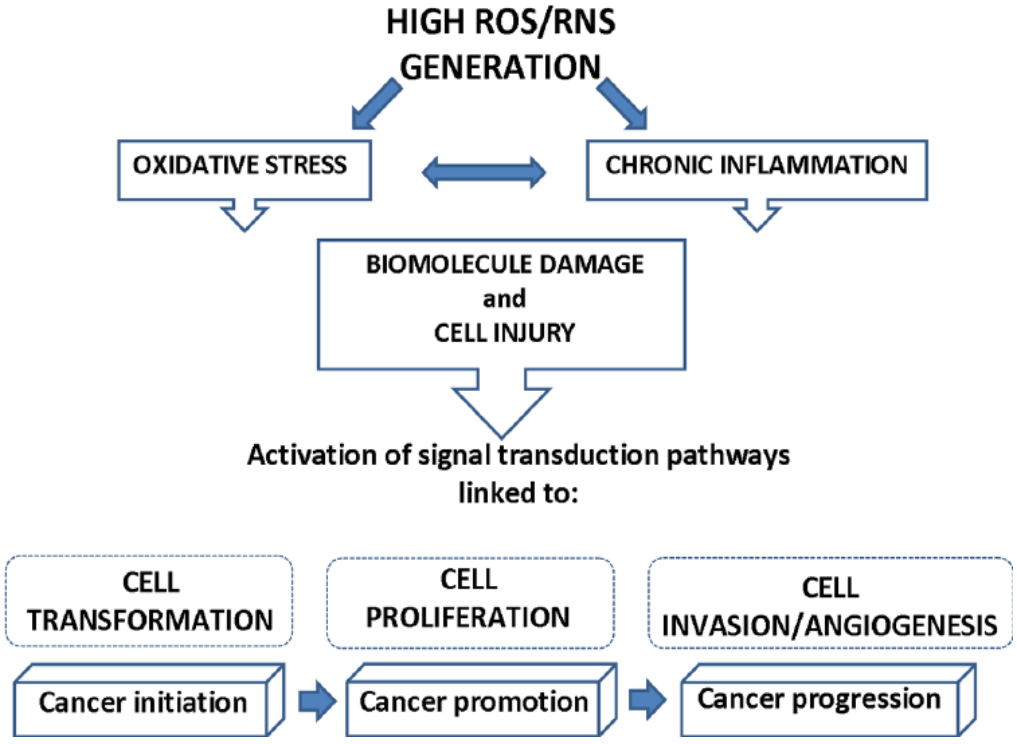

Figure 4. Diagram of the association between ROS/RNS generation, oxidative stress, inflammation and carcinogenesis. Excessive ROS and RNS, leads to oxidative stress and chronic inflammation. Benedetti S, et al. Reactive oxygen species a double-edged sword for mesothelioma. *Oncotarget* 6(19), 2015.

Inflammatory cells are particularly effective in generating most of the reactive oxygen species. The activation of the redox metabolism of the inflammatory cells generates a highly oxidative environment within an organ of aerobic organisms, especially lungs which are exposed to breating air on a constant daily basis. Much of the oxygen biochemistry, through the activation of plasma membrane NADPH oxidase, of macrophages and neutrophils, is directed towards the release of superoxide anion ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals $HO^{\bullet}$).[61-63]

The antagonism between inflammation and immunity also affects the outcome of cancer treatment. Scientists noticed that cancer development and its response to therapy are strongly influenced by innate and adaptive immunity. Clinical studiers observed that chronic inflammation promoted tumour development, progression, and metastatic dissemination, which affect treatment by anticancer drugs. Cancer development and malignant progression are also associated with accumulation of genetic alterations and loss of normal regulatory processes, which cause expression of tumour-specific antigens and tumour-associated antigens which can activate antitumour immune responses.[64]

In the last decade, the immune chekpoint therapy, which targets regulatory pathways in T cells to enhance antitumour immune responses, had led to revolutionary clinical advances and new drugs for the fight against cancer. This is a result of many years of studies of how the human immune system responds to tumour microenvironment. Professors James P. Allison and Tasuku Honjo were awarded the 2018 Nobel Prize for Physiology or Medicine for their discovery of cancer therapy by inhibition of negative immune regulation. A new principle for cancer therapy by unleashing the human immune system to attack cancer cells.[65-67]

Extensive research during the past decades has revealed the mechanism by which continued oxidative stress can lead to chronic inflammation, which in turn could mediate most chronic diseases including pulmonary cancer and cardiovascular diseases. Oxidative stress can activate a variety of transcription factors including the a-g types, which play crucial roles in cancer initiation and promotion:[68,69]

a. NF-κB (nuclear factor, induces the expression of anti-apoptotic genes),
b. AP-1 (Activator protein**,** controls a number of cellular processes including differentiation, proliferation, and apoptosis),

c. p53 (is a tumor suppressor gene),
d. HIF-1α (Hypoxia-inducible factor 1-alpha),
e. PPAR-γ (Peroxisome proliferator-activated receptor gamma),
f. Wbt/β-catenin (signaling pathway, essential role during development and adult tissue homeostasis), and,
g. Nrf2 (nuclear factor erythroid 2–related factor 2, an emerging regulator of cellular resistance to oxidants).

Activation of these transcription factors can lead to the expression of over 500 different genes, including those for growth factors, inflammatory cytokines, chemokines, cell cycle regulatory molecules, and anti-inflammatory molecules. Studies showed that oxidative stress activates inflammatory pathways leading to transformation of a normal cell to tumour cell, tumour cell survival, proliferation, chemoresistance, radioresistance, invasion, angiogenesis, and stem cell survival. Overall, observations to date suggest that oxidative stress, chronic inflammation, and cancer are closely linked.[70-72]

A range of inflammation mediators, including cytokines, chemokines, free radicals, prostaglandins, growth and transcription factors, microRNAs, and enzymes as, cyclooxygenase and matrix metalloproteinase, collectively acts to create a favourable microenvironment for the development of tumours. The possible mechanisms by which inflammation can contribute to carcinogenesis include genomic instability, alterations in epigenetic events and subsequent inappropriate gene expression, enhanced proliferation of initiated cells, resistance to apoptosis, aggressive tumour neovascularization, invasion through tumour-associated basement membrane and metastasis.[73,74]

Cancer-associated inflammation is also linked with immune-suppression that allows cancer cells to evade detection by the immune system. Studies observed that the tumour microenvironment, which is largely orchestrated by inflammatory cells, is an indispensable participant in the neoplastic process, fostering proliferation, survival and migration. In addition, tumour cells have co-opted some of the signalling molecules of the innate immune system, such as selectins, chemokines and their receptors for invasion, migration and metastasis. These insights are fostering new anti-inflammatory therapeutic approaches to cancer development.[75-78]

Tumour growth and metastasis depend on angiogenesis and lymphangiogenesis triggered by chemical signals from tumour cells in a phase of rapid growth. Pathological angiogenesis, which is a hallmark of cancer progression and various ischaemic and inflammatory diseases, is associated with chronic inflammation. Both inflammation and angiogenesis are exacerbated by increased production of chemokines/cytokines, growth factors, proteolytic enzymes, proteoglycans, lipid mediators and prostaglandins. Different proteins have been identified as angiogenic activators, including vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), angiogenin, transforming growth factor (TGF)-α, TGF-β, etc. It has been reported that approximately 15–20% of all malignancies are initiated or exacerbated by inflammation. The stages of Initiation and progression of cancer are also closely linked to angiogenesis. Infiltration of macrophages is a dramatic and common feature of inflammation, angiogenesis and cancer, and has been recently highlighted in an attempt to develop novel strategies for treating cancer.[79-82]

Exposure to aiborne particulate matter, ROS and oxidative stress

The main current paradigm in airborne inhalable particular matter (PM) toxicology is centred on the concept of oxidative stress. The ability of respirable particles or fibrous dusts to enter the respiratory system and penetrate deep into the lung's alveoli in order to generate free radicals or ROS is suggested to be the main factor involved in their pathogenic potential. There is abunadance of scientific evidence that PM through the production of ROS are involved in cellular damage, especially in lipid peroxidation, DNA double helix breaks and mutations and protein oxidative damage. Inhalable PM fractions have different oxidative effects. Respirable particles (PM_{10}, $PM_{2.5}$ in μm of aerodynamic diameter) generate hydroxyl radical ($HO^{\bullet}$) through redox reactions. Most of these radicals are the result of redox mechanisms by heavy metals adsorbed on the pores and surfaces of the particles. Larger respirable particles deposit mainly in the upper airways and can be cleared by the mucociliary system. [83,84]

Research on PM focused on the ultrafine particles (UFPs) with diameter less than 100 nm; because of their very high alveolar deposition fraction, large surface area, chemical composition (carbonaceous stable radicals), and ability to enter into the blood circulation and induce inflammation. In addition, the results indicate that PM-mediated ROS production is involved in the generation of inflammation and activated inflammatory cells can increase their ROS production.[85,86]

Oxidative stress is the responsible factor for the rise of pulmonary pathology through airway inflammation, particularly when the human respiratory system is exposed to inhalable airborne particles, mostly from ambient air pollution, petrol and diesel fumes in motorways.[87]

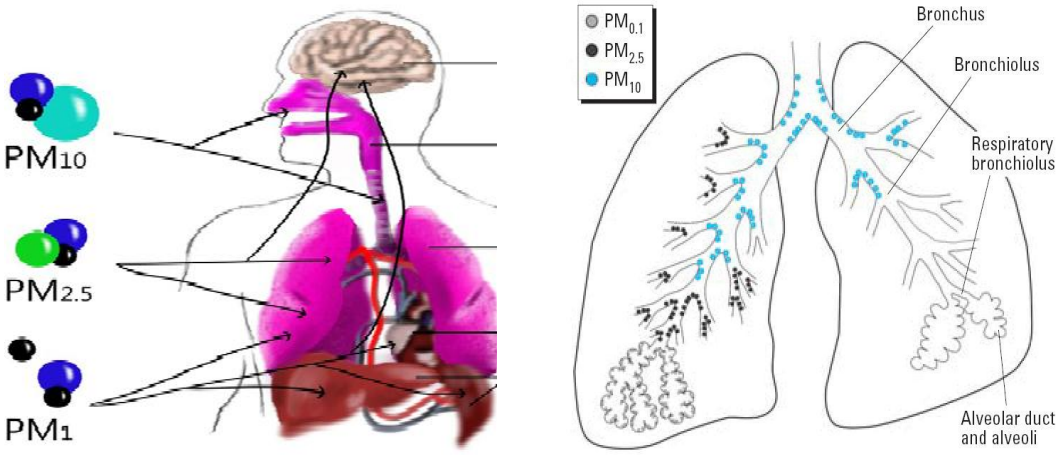

Figure 5. Generation of oxidatively damage by particulate matter (PM) has been established by scientific research to occur via production of reactive oxygen species (ROS) and inflammation in the respiratory bronchiolus and alveoli.
[Cornier S, Lomnicki S, Backes W, Dellinger B. Origin and health impacts of emissions of toxic by-products and fine particles from combustion and thermal treatment of hazardous wastes and materials. *Environ Health Perspect* 114(6):810-817, 2006].

A study by Chinese scientists investigated the *in vitro* toxicity of $PM_{2.5}$ collected at six urban sites in China, and how particle composition affects their cytotoxic mechanisms through the production of ROS. For the experimental section they used human bronchial epithelial (BEAS-2B) cell lines as model *in vitro* to expose to $PM_{2.5}$ and analyzed the production of ROS, superoxide dismutase activity and total antioxidant capaciy. The results showed that high concentrations of polycyclic aromatic hydrocarbons (PAHs) and elemental Nickel (Ni) were strongly associated with high apoptosis rates and high expression of IL-1β. They noticed that addition of

Fe element was associated with the ROS level, and furthermore, Fe and Cr element were associated with DNA damage.[88]

Another recent study examined the impact of repeated exposures to air pollution by using urban particulate matter (PM) on mouse lungs with focus on inflammatory and oxidative stress parameters. Aqueous extracts from collected urban PM were administered to mice by 5 repeated intra-tracheal instillations. Repeated exposure to PM caused systemic inflammation and oxidative damage to lung tissue lipids and proteins through mechanisms of ROS. Multiple exposures, led to an increase in cytokine levels in both bronchoalveolar lavage fluid and in the blood serum, indicating a systemic reaction. Lung mRNA levels of antioxidant/phase II detoxifying enzymes decreased by exposure to the PM extract, but not when metals were removed by chelation. Redox metals are known to be responsible for the production of ROS. Disruption of lung tissue oxidant-inflammatory/defense balance was evidenced by increased levels of lipid and protein oxidation.[89]

A number of experimental studies established that airborne PM, like tobacco smoke, are responsible for producing ROS that have been implicated in the activation of mitogen-activated protein kinase (MAPK) family members and activation of transcription factors such as NF-κB and AP-1 (the activator protein 1). These signalling pathways have been implicated in processes of inflammation, apoptosis, proliferation, transformation and differentiation.[90,91]

Airborne PM of urban air pollution (mainly exhaust fumes of diesel and petrol vehicles) represents a mixture of many different chemical components that consists of a variable carbonaceous particle core (with surface pores absorbing metallic elements, Fe, Cd, Pb, Sr, Sn, Va and Zn) and a large array of surface-bound constituents including PAHs, redox heavy metals and stable quinoid free radicals, releasing continuously the highly rective hydroxyl radical.[92-94]

Fine respirable particles (<$PM_{2.5}$) penetrate deep into the lung's alveoli and act in a synergistic mechanism with other components of air pollution (O_3, NO_x, soot, heavy metals, PAHs). Synergy of ambient PM and tobacco smoke has been studied. Smokers in urban areas showed much higher risk for lung cancer than non smokers. The porous surfaces of carconaceous airborne particles (e.g. PM from diesel engine combustion fumes) provide a fertile ground for absorption and catalyze the

increased generation of ROS or other damaging oxidants which are potential initiators of pulmonary carcinogenesis. Synergistic effects for increased ROS formatiomn have been experimentally verified. Also, the alteration of intracellular calcium homeostasis induced by $PM_{2.5}$ is closely correlated to an increase of oxidative stress.[95,96]

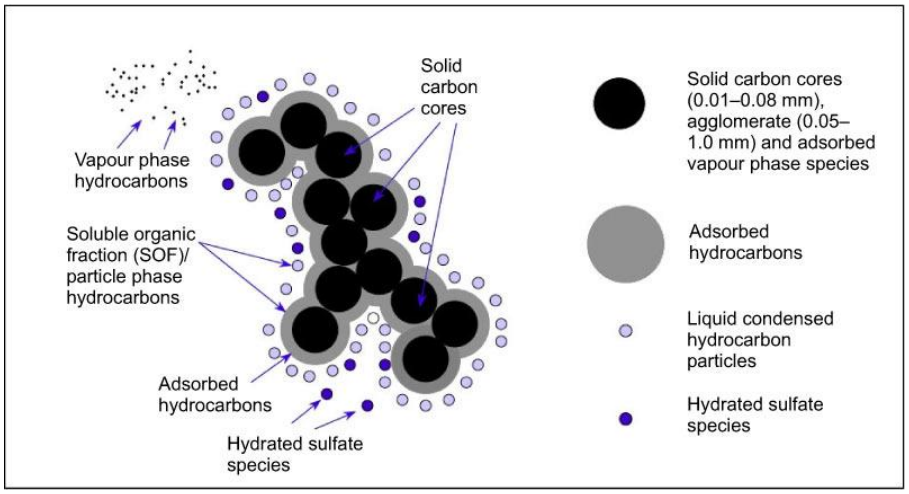

Figure 6. Carbonaceous fine particulate matter (PM) has porous surfaces which can adsorb other chemicals and metals, catalysing the production of ROS. PM can be from diesel engine combustion exhausts [Twigg M, Phillips P. Cleaning the air we breathe-controlling diesel particulate emission from passenger cars. *Platinum Metals Rev* 53(1):27-, 2009].

Synergistic or additive effects (from synergy) are the result between two or more agents, or substances that produce an effect greater than the sum of their individual effects. Synergy is the opposite of antagonism where agents act in contrasting actions or opposing effects. Particles with their porous surfaces and adsorbed metals act in a synergistic manner with other gaseous or semivolatile pollutants. Also, stable free radicals (semiquinones) in the core of particles generate radicals.[97] Environmentalists and toxicologists in the U.S. EPA and W. Europe are shifting their studies toward a multi-pollutant approach to quantify the health consequences of air pollution mixtures as a whole, while recognizing that such a paradigm shift will be challenging because of the effects of synergy.[98]

It has been observed with detailed free radical experiments (Electron Paramagnetic Resonance) that Increasing ROS generation is the result of synergy between ambient transition metals and PM semiquinoid stable radicals (in the core

of carbonaceous particles), PAHs of airborne particles and tobacco smoke, ambient NO_X and cigarette tar which is similar to PM composition.[99-101]

Synergy for increasing ROS production occurs between coarse carbon particles (soot) and iron particles (Fe), PM_{10} and ozone, mineral fibres and tobacco smoke and between PM and wood smoke.[102-106]

Defense response of the respiratory tract to xenobiotics invasion

The respiratory tract is a complex organ system with many different functions and defense mechanisms throughout the nasopharyngeal, tracheobronchial, and pulmonary regions. Exposure to xenobiotics (particles, gas pollutants, microorganisms) by inhalation initiates various protective mechanisms. Mucociliary clearance is the work of cilia, tiny muscular, hair-like projections on the cells that line the airways, are one of the respiratory system's defense mechanisms. Alveolar macrophages, a type of white blood cells (innate immune system) on the surface of alveoli, are another defense mechanism, with the purpose of clearing the air spaces of infectious, toxic, or allergic particles that have evaded the respiratory system.

Reactive oxygen species (ROS) can be generated by target cells such as lung epithelial cells and pulmonary macrophages upon interaction with and/or uptake of airborne particulate matter (PM) and air pollutants. Alveolar macrophages have an important role in clearing xenobiotic particles from the lungs and in response they can release ROS. Phagocytic cells of the innate immune system such as alveolar macrophages and polymorphonuclear neutrophils are highly proficient producers of ROS to enhance microbiocidal conditions in phagocytic vacuoles and eliminate pathogenic bacteria or potentially harmful particles. Air pollution particles cause direct activation of alveolar macrophages to produce ROS part of which are prevented by antioxidants circulating in the blood.[107,108]

Resident macrophages in the airways and in the alveolar spaces can release ROS/RNS after phagocytosis of inhaled particles. These macrophages also release large amounts of TNF-alpha (Tumour necrosis factor-alpha), a cytokine that can generate responses within the airway epithelium dependent upon intracellular generation of ROS/RNS. As a result, signal transduction pathways are set in motion

initiating inflammation and other pathobiology pathways in the lung airways. Chemokines have been shown to regulate inflammation and immune cell differentiation in respiratory diseases. Such effects include increased expression of intercellular adhesion molecule 1, interleukin-6, cytosolic and inducible nitric oxide synthase, manganese superoxide dismutase (MnSOD), cytosolic phospholipase A2, and hypersecretion of mucus. Ultimately, ROS/RNS may play a role in the response of the airway epithelium to particulate pollutants via activation of kinases and transcription factors common to many response genes. Thus, defense mechanisms involved in responding to offending particulates may result in a complex cascade of events that can finally contribute to airway pathology.[109-111]

In a chronic pulmonary inflammatory setting, persistent production of ROS/RNS can cause considerable tissue damage. People with chronic inflammatory diseases are more susceptible for adverse health effects in the lungs by exposure to airborne PM in urban settings. In turn oxidative stress and inflammation in the pulmonary tissues are crucial factors for DNA strand breaks and DNA oxidation, factors that are implicated in the initiation stage of carcinogenesis. A substantial number of toxicological studies consider that the generation of ROS in pulmonary cells is one of the most important mechanism for lung carcinogenesis.[112,113]

Studies in the last decade have established that ROS as a group of highly reactive molecules, which act as powerful signaling molecules, are involved in the regulation of various biological processes. For instance, cancer cells have increased ROS levels in comparison to their normal counterparts, due to an enhanced metabolism and mitochondrial dysfunction and contribute to the biochemical changes necessary for the tumour initiation, promotion and progression. This role of ROS is being delineated continuously and becoming pronounced, adding complexity of these radicals in understanding of their pathophysiology. Scientific investigations proved that ROS, depending on the concentration and duration of exposure, can damage cellular proteins, mebrane lipids, and DNA, leading to genomic instability and activation of various signaling cascades related to tumorigenesis. DNA damage, leading to activation of oncogenes or inactivation of tumour suppressor genes, is one of the plausible mechanisms by which ROS can promote carcinogenesis.[114,115]

Inhalation of fibrous dusts and O_3

Inhalable inorganic particulates, silica and quartz dust, coal mine dusts, asbestos and other fibrous minerals are some of the etiological agents for the production of ROS in the respiratory tract, contributing to oxidative stress and inflammation. Also, ozone (O_3) and nitrogen oxides (NO_x) are air pollution oxidants contributing to oxidative stress in the lungs. Exposure of the respiratory system to these agents stimulate alveolar macrophages or bronchial epithelial cells to release chemotactic factors that recruit inflammatory cells to the lung.[116,117]

The human respiratory system has developed effective antioxidant defenses to be able to deal with oxidative damage from particles. The mucociliary escalator in the airways traps and sweeps particles out of the lungs, whereas the alveolar macrophages phagocytose particles and then remove them to the gut. Problems develop when excessive oxidative stress damages macrophages and stimulates the release of inflammatory mediators from macrophages or epithelial cells and cell signaling is associated with the production of ROS. Cellular signaling induced by inhalable particles and O_3 exposure varies with cell type and physiochemical properties of these pollutants. Cellular signaling plays a critical role in the regulation of inflammatory pathogenesis.[118]

In the last decades numerous studies were performed with inhalable particlers, dusts and fibrous inorganic materials in experimental animals. Persistent inflammation and associated excessive oxidative stress in the rat lungs have been crucially implicated in quartz-induced pulmonary diseases, including fibrosis and cancer.[119] At intermediate levels, oxidative stress activates mitogen-activated protein kinases (MAPKs) and central pro-inflammatory transcription factors such as NF-κB and AP-1 leading to up-regulation of pro-inflammatory genes.[120] Experiments with rat alveolar macrophages showed that exposed to coarse chalk dust particles resulted in respiratory burst and oxidative stress. The rsult was the production of ROS and RNS in the alveolar mactophages.[121]

Nanotechnology and numerous nanomaterials have been introduced in the last decade into many scientific and technological areas (biology, medicine, fiber optic communication networks, aerospace technology, advanced materials

technology, chemical engineering and precision manufacturing). In the last years vast amounts of engineered nanomaterials (ENMs) have been produced but the health and safety aspects of ENMs are still in its formative stage. Respiratory system toxicity from inhalable ENMs is the most important concern to health specialists. Scientific evidence has shown that there is a close connection between respirable ENMs and pulmonary oxidative stress through the generation of ROS/RNS and connection between oxidative stress in the cell with elicitation of an inflammatory response via pro-inflammatory gene transcription. Studies *in vitro* and *in vivo* have shown that ENMs at various doses can cause ROS generation, oxidative stress, and pro-inflammatory gene expression in the respiratory system.[122,123]

Experiments with exposure of human lung epithelial cells to enginneered nanoparticles showed that there was an additional role of nanoparticles as carriers of heavy metals. Results indicated that the nanoparticles could efficiently enter the cells by a Trojan-horse type mechanism which provoked an up to 8 times higher oxidative stress in the case of cobalt (Co) or manganese (Mn) if compared to reference cultures exposed to aqueous solutions of the same metals. The presence of catalytic activity in the mertallic component of nanoparticles could strongly alter their damaging oxidative action.[124]

Nitrogen oxides (NO, NO_2) and ozone (O_3) are ubiquitous air toxicants capable of inducing lung damage to the respiratory epithelium. Primary human bronchial epithelial cells were exposed for 2 hours at an air-liquid interface to 3 ppm NO_2, 0.75 ppm O_3, or filtered air. Gene expression was measured using PCR arrays for toxicity and oxidative stress. The results showed that genes related to oxidative stress were highly induced with NO_2 while expression of pro-inflammatory and vascular function genes was found with subsequent exposure to O_3.[125]

Pulmonary oxidative stress, inflammation and oxidative DNA damage

The increased presence of oxygen in the Earth's atmosphere produces a more efficient energy source in mitochondria (aerobic metabolism producing 16–18 times more adenosine triphosphate (ATP) per hexose sugar than anaerobic metabolism.[126,127] Aerobic organisms are in a constant daily struggle to minimize and

then repair the results of oxidatively induced cellular damage. Multicellular eukaryotic life forms evolved enzymatic and non-enzymatic defense mechanisms to withstand oxidative damage in proteins and DNA-RNA-mtDNA.[128,129]

Endogenous and exogenous oxidative damage to cellular (DNA-RNA) and mitochondrial DNA (mtDNA) in aerobic organisms is an everyday occurence and is accepted as a bilogical experimental fact which can me measured quantitatively. It has been estimated that one human cell is exposed daily to approximate 10^3-10^5 oxidative hits by the hydroxyl radical ($HO^{\bullet}$) alone. The highly reactive hydroxyl radical reacts with the heterocyclic DNA nucleobases and the sugar moiety near or at diffusion-controlled rates leading to adduct radicals.[130-132]

The accumulation of oxidative damage to cellular and mitochondirial DNA in aerobic organisms has been established as important causative factor of a great range of human diseases and aging. These oxidative lesions were investigated in the past several decades and the results suggested that the oxidatively induced DNA adducts, especially bulky DNA lesions, may serve as biomarkers for exploring the role of oxidative stress in human diseases.[133]

The mitochondrial DNA (mtDNA) is very gene dense and encodes factors critical for oxidative phosphorylation, but it is exposed to greater oxidative damage due to the “mistakes” that occur during the production of ATP through the electron transport chain. Mutations of mtDNA cause a variety of human mitochondrial diseases and are also heavily implicated in age-associated diseases, such as cancer, diabetes, cardiovascular, neurodegenerative, and aging. There has been considerable progress in understanding the role of mtDNA mutations in human pathology during the last two decades.[134]

Studies have shown that the mitochondrion is a sensitive target of both oxidative stress and environmental pollutants, like ultrafine particulate matter with aerodynamic diameter 2.5 micrometers, μm, ($PM_{2.5}$) which penetrate deep in the lung alveoli.[135] Scientists have contacted experiments with PM exposure during pregnancy of mother and newborne. Results of these studies showed that exposure to airborne PM is associated with mitochondrial DNA damage during pregnancy in both mothers and their newborne. The study observed that particulate air pollution

exposure in early life plays a role in increasing systemic oxidative stress, at the level of the mitochondria, both in mother and foetus.[136]

A recent review collected relevant studies for the interaction between ambient particulate matter (PM), and genomic instability in biological organisms and their fundamental role in lung carcinogenesis. According to epidemiological and toxicological evidence, PM acts as a carcinogenic factor inducing high rates of genomic alterations or damages. These genomic damages are capable of acting as a driving force of the carcinogenic process. The frequency of these alterations is related to the loss of fidelity in mechanisms such as DNA replication, chromosomal segregation, DNA repair and cell-cycle progression.[137]

Biomonitoring studies in humans have shown associations between exposure to air pollution particles and high rates of oxidative damage to DNA. The elucidation of carcinogenic mechanisms by oxidative DNA damage incidate at least two different pathways thought to play an important role. The first mechanism is thought to act through the modulation of gene expression affecting intracellular signaling pathways, whereas the second is thought to proceed through the induction of genetic damage (mutations, strand breaks, chromosomal rearrangements) and a blockage to the DNA replication.[138-140]

One of the most important and widely studied biomarker of oxidative DNA damage caused by reactive ROS/RNS is the adduct of hydroxyl radicals ($HO^{\bullet}$) on DNA nucleobases, especially deoxynucleosides. The 8-hydroxy-2'-deoxyguanosine (8-OHdG) and/or its tautomeric 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) has been proved an important mutagenic adduct to DNA and therefore a potential biomarker of carcinogenesis. Mutations of 8-oxodG involve a GC → TA transversion.
In nuclear and mitochondrial DNA, 8-hydroxy-2'-deoxyguanosine (8-OHdG) is the most frequently detected and studied DNA lesion which is excreted in the urine. The 8-OHdG is not only a biomarker of oxidative stress but also can be used as a risk factor for cancer, atherosclerosis and diabetes. Elevated level of urinary 8-OHdG has been detected in patients with various cancers. In the last decade 8-hydroxydeoxyguanosine (8-OHdG) is a commonly used marker of DNA oxidative stress in epidemiological studies.[141-143]

Various biomarkers in urine samples of oxidative stress, such as 8-hydroxy-2'-deoxyguanosine (8-OHdG), malondialdehyde (MDA, a biomarker of lipid peroxidation), 8-isoprostane (8-IsoP, a bioactive metabolite resulting from the peroxidation of arachidonic acid) and Vascular Endothelial Growth Factor (VEGF), are used regularly in many studies for quantitative measurements of exposure ambient air pollutant levels.[144,145] A recent study found a statistical significance correlation between the levels of 8-OHdG and smoking index. The quantitative determination of 8-OHdG showed a sensitivity and specificity (70% and 73.7%) to identify a patient with lung cancer and suggest that 8-OHdG is a potential diagnostic biomarker for lung cancer.[146]

Multistep progression in lung carcinogenesis

The accumulation of experimental, clinical and epidemilogic studies on the mechanisms of lung carcinogenesis, led scientists to agree on the crucial stages leading to initiation, promotion and progression of malignant neoplasms in the lungs. The initiation starts with the production of ROS by carcinogens and other risk factors. This is followed by oxidative stress as a result of depletion of antioxidant defenses, leading to inflammatory processes that have both genotoxic implications, and activation of transcription factors with important role in carcinogenesis. Lung carcinogenesis is a complex, stepwise process that involves the acquisition of genetic mutations and epigenetic changes that alter cellular processes, such as proliferation, differentiation, invasion, and metastasis.[147]

Finally, to conclude the review I included a short selection of lung cancer studies with exposure to particulate matter (MP), asbestos fibres and tobacco smoking (major lung cancer causes), which explain the contribution to carcinogenic mechanisms for the multistep progression to malignant neoplasm.

A recent study focused on the basic concepts on lung cancer mechanisms triggered after exposure to combustion derived particular matter (PM). Experimental evidence points to Inflammation caused by PM in the respiratory system. The results have both genotoxic and non-genotoxic implications that play a central role in development of various health outcomes, including cancer. Chronic, low-grade

inflammation may cause DNA damage through a persistent increased level of reactive oxygen species (ROS) produced and released by activated immune cells. At the same time a number of pro-inflammatory cytokines and chemokines display mitogenic, motogenic, morphogenic and/or angiogenic properties and therefore contribute to tumour growth and metastasis.[148]

The key triggering events involved in activation of pro-inflammatory responses by Combustion PM (CPM) and soluble CPM components can be categorized into (i) formation of ROS and oxidative stress, (ii) interaction with the lipid layer of cellular membranes, (iii) activation of receptors, ion channels and transporters on the cell surface and (iv) interactions with intracellular molecular targets including receptors such as the aryl hydrocarbon receptor (AhR). These categories of mechanisms are able to elucidate the effects of diesel exhaust particles (DEP) using human lung epithelial cells as a model system. Diesel exhaust particles from vehicles are considered highly carcinogenic in the lungs.[149]

Malignant mesothelioma (MM) is a relatively rare type of pulmonary cancer that occurs almost exclusively following respiratory exposure to asbestos in humans. It is well known that in the past asbestos miners in South Africa (global leader in asbestos production) died in great numbers by mesothelioma (more than 2,700 in 2002). Asbestos fibers' carcinogenesis is closely associated with iron overload and oxidative stress in mesothelial cells. Asbestos fibers catalyze free radical generation, leading to oxidative DNA damage, followed by activation of intracellular signaling pathways. Studies showed that exposure to asbestos fibers induce chronic inflammation, involving consistent generation of free radicals that contribute to mesothelial carcinogenesis. Recent mechanistic studies identified the critical role of chronic inflammation in promoting mesothelioma growth.[150,151]

The carcinogenicity of tobacco smoke has been recognized by numerous investigations and carcinogenic mechanism studies *in vitro* and *in vivo*. Cigarette smoking is not only a risk factor to lung cancer but for many inflammatory diseases leading to a number of malignances, such as esophagus, larynx, mouth, throat, kidney, bladder, liver, pancreas, stomach, cervix, colon, rectum, as well as acute myeloid leukemia. Recent advances in molecular biology and immunology have improved the knowledge on different mechanisms implicated in lung cell malignant

transformation, progression and metastasis, thus presenting a promissing new era for lung anticancer therapies. Tobacco smoke induces lung malignancy by a large number of different mechanisms and carcinogenic substances in the cigarette tar and the gases/volatile chemicals in the smoke. Studies identified numerous carcinogenic and mutagenic compounds in cigarette smoke which can induce direct cytotoxicity and/or mutagenic action on lung epithelial cells. These damages can be achieved by generation of somatic mutations, epigenetic events, epithelial cell to mesenchymal cell transformations, as well as by chronic cell damage. Tobacco smoke induces chronic lung inflammatory microenvironment, oxidative stress and cell structural alterations such as the increase of cell proliferation, angiogenesis and apoptosis arrest. All these mechanisms are irreversible processes that have a high influence in lung tumour growth.[152-154]

Conclusion

Lung cancer is a malignant lung disease primarily caused by cigarette smoking. Lung cancer in humans is fatal in short period of time and the overall survival rate is estimated from studies to be about 17% at five years after diagnosis. The low rate of survival of patients from lung cancer is the result of rapid spread (metastasis) to other parts of the body. Recent epidemiological investigations have shown associations between increased incidence of respiratory diseases and lung cancer from exposure to tobacco smoke and various forms of respirable mineral fibers (such as asbestos, silica, metal oxidesm etc) and ambient air pollution particulate matter (PM), at occupational or urban air polluting environments. Substantial number of studies showed that the generation of reactive ogygen species and nitrogen species (ROS/RNS), leading to oxidative stress and inflammation with high DNA damage play an important role in intitiation and progression mechanisms of lung carcinogenesis. Although tobacco smoke, active and passive smoking, is a major factor of lung cancer, inhalable particles, inorganic dusts, asbestos fibers and vehicular exhausts have a substantial contribution to respiratory diseases and in particular lung cancer. These factors can act synergistically in the respiratory system and increase substantially the production of ROS causing an imbalance between

oxidants and antioxidants in the respiratory tissues. In turn oxidative stress can damage cellular proteins, mebrane lipids, and DNA, leading to genomic instability and activation of various inflammatory signaling cascades related to lung carcinogenesis. Scientists suggest that immunosuppression may contribute to lung carcinogenesis by allowing lung cancer cells to escape immune surveillance. Tumour cells may contribute to immunosuppression by releasing suppressive cytokines, augmenting the trafficking of suppressor cells to the tumour site. Despite the numerous discoveries related to mechanisms and factors of pulmonary carcinogenesis, lung cancer is extremely complex cancer type and prevents the effective therapeutic actions. This is the reason that mortality from lung cancer remains higher than that of any other types of cancer. Scientists focused on the evolving concept that inflammation in the lungs sets up a field of injury that promotes the development of lung cancer and that the entire epithelium is involved in the stepwise progression to lung cancer. Research showed that reparative cells (cells that effecting repair) in the field of cancerization represent tumour-initiating cells, which develop additive and sometimes synergistic molecular changes that result in stepwise progression to lung cancer. Research has to elucidate the role of tumour-initiating cells and their aberrant signaling pathways, as do the specific genetic and epigenetic alterations in these cells that provide the irreversible event for the development tumours.

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