

Oxidative Stress and Changed Gene Expression Profiles in Fiber-/Particle-Induced Carcinogenesis

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ABSTRACT Exposure to ambient air pollution (particles, fibres) is associated with pulmonary diseases and cancer. The mechanisms of induced health effects are believed to involve inflammation and oxidative stress. Oxidative stress mediated by airborne particles and/or fibres may arise from direct generation of reactive oxygen species (ROS) from the surface of particles/fibres, soluble compounds such as transition metals or organic compounds, and activation of inflammatory cells capable of generating ROS and reactive nitrogen species (RNS). Generation of ROS/RNS can directly cause covalent modifications to DNA or they can initiate the formation of genotoxic lipid hydroperoxides. The resulting oxidative DNA damage can lead to changed gene expression such as upregulation of tumor promoters and downregulation of tumor suppressor genes and thus may be implicated in cancer development. The present review describes the important role of free radicals in particle-/fibre- induced cellular damage, the interaction of ROS with target molecules, especially with DNA, and the modulation of specific genes and transcription factor caused by oxidative stress.

ROLE OF OXIDANTS IN PARTICLE-/ FIBRE-INDUCED CELLULAR DAMAGE

General Mechanisms of Oxidative Stress

Oxidative stress is a general term used to describe the steady state level of oxidative damage in a cell, tissue, or organ, caused by reactive oxygen and reactive nitrogen species (ROS/RNS). ROS, such as free radicals and peroxides, represent a class of molecules that are derived from the metabolism of oxygen and exist inherently in all aerobic organisms (Halliwell and Gutteridge 1989). There are many different sources through which ROS are generated. Under normal conditions, ROS is produced from the endogenous sources as by-products of normal and essential metabolic reactions, such as energy generation from mitochondria or the detoxification reactions involving the liver cytochrome P-450 enzyme system (Proctor and Reynolds 1984). Exogenous sources include exposure to airborne substances such as particles like metals (ferric, copper, nickel, cadmium, etc.), and fibrous minerals like

asbestos, as well as, metal(loid)s such as arsenic and barium to which people are exposed after their intake via polluted water or carrier particles. Beside acting as carcinogens, some of these airborne substances act as co-carcinogens in association with several other well known carcinogens like cigarette and biofuel smokes along with other types of exposures like UV and X-ray, etc. enhancing their effects many folds. Normal exposure to airborne substances like particles and fibers in the lungs is followed by a chain of reactions, which starts with a change in the free cell population, including pulmonary macrophages and polymorphonuclear inflammatory cells via their influx (Spruzem et al. 1987). It is believed that mineral dusts are transported from the alveolar epithelium into the lung interstitium by these inflammatory cells (Brody et al. 1981), which, after stimulation with the foreign fibers/particles produce ROS, RNS and growth factors (Cohen 1981) further stimulating fibroblasts to produce collagen and causing fibrosis of lungs.

Initial reactions by the cells under normal conditions through metabolic processes as well as in exposed conditions following oxygen activation, is the production of superoxide anions also known as "primary ROS". This superoxide anion is formed by the univalent reduction of triple – state molecular oxygen ($^3\text{O}_2$). These superoxide anions further interact with other molecules to generate "secondary ROS", either

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directly or indirectly via interaction with enzymes or metal catalysed processes (Halliwell and Cross 1994).

Normally, the direct interaction of the superoxide anions with the biological molecules such as sugars, nucleotides, polypeptides is controversial (Valko et al. 2006). Superoxide undergoes reaction with the enzyme superoxide dismutase (SOD) to get converted into hydrogen peroxide and singlet oxygen.



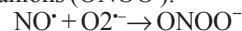
This hydrogen peroxide is then removed with enzymes such as catalases and glutathione peroxidases (Michiels et al. 1994). These enzymes convert this H_2O_2 to harmless water molecules. However, this redox state is largely dependent on the quantity of transition metals in the surrounding (e.g. cuprous and ferrous ions). If these metals are present in high amounts, they cause the conversion of the hydrogen peroxide to highly reactive hydroxyl radicals ($\text{OH}^\cdot$) (Chance et al. 1979). Excessive presence of superoxide also leads to the release of additional “free iron” from the iron containing molecules leading to the increase of a labile iron pool within the cell, which represents the amount of exchangeable and chelable iron (Kakhlon and Cabantchik 2002). This has been demonstrated for [4Fe-4S] cluster-containing enzymes of the dehydratase-lyase family (Liochev and Fridovich 1994). The released Fe(II) further participates in the Fenton reaction, generating highly reactive hydroxyl radicals ($\text{Fe(II)} + \text{H}_2\text{O}_2 \rightarrow \text{Fe(III)} + \text{OH}^\cdot + \text{OH}^-$). Thus, under stress conditions, $\text{O}_2^{\cdot -}$ acts as an oxidant of [4Fe-4S] cluster-containing enzymes and facilitates $\text{OH}^\cdot$ production from H_2O_2 by making Fe(II) available for the Fenton reaction (Leonard et al. 2004a; Liochev and Fridovich. 2002; Pekarkova et al. 2001). The superoxide radical participates in the Haber-Weiss reaction ($\text{O}_2^{\cdot -} + \text{H}_2\text{O}_2 \rightarrow \text{O}_2 + \text{OH}^\cdot + \text{OH}^-$) which combines a Fenton reaction and the reduction of Fe(III) by superoxide, yielding Fe(II) and oxygen ($\text{Fe(III)} + \text{O}_2^{\cdot -} \rightarrow \text{Fe(II)} + \text{O}_2$) (Pastor et al. 2000).

Production of $\text{OH}^\cdot$ close to DNA could lead to this radical reacting with DNA bases or the deoxyribosyl backbone of DNA to produce damaged bases or strand breaks. It has been proposed that the extent of DNA strand breaking by $\text{OH}^\cdot$ is governed by the accessible surface areas of the hydrogen atoms of the DNA backbone. Typical additional radicals derived

from oxygen that can be formed in living systems and can damage DNA/RNA are peroxy radicals ($\text{ROO}^\cdot$). Peroxy radicals are high-energy species, with a reduction potential ranging from +0.77 to +1.44V, depending on the R group (Burcham 1998). The simplest peroxy radical is the dioxy (hydroperoxy) radical $\text{HOO}^\cdot$, which is the conjugate acid of superoxide, $\text{O}_2^{\cdot -}$. The chemistry of this type of molecule varies according to the nature of the R group, the local environment, and the concentration of oxygen and of other reactants (Porter et al. 1995). Peroxy radicals are involved in DNA cleavage and protein backbone modification. Peroxy radicals synergistically enhance the induction of DNA damage by superoxide (Valko et al. 2006).

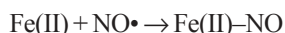
In airways endogenous Nitric Oxide ($\text{NO}^\cdot$) plays a key role in the physiological regulation of important paracrine and autocrine functions like relaxation of smooth muscle in arterioles, inhibition of platelet aggregation and inflammation, effects on secretion and the autonomic nervous system. Reported effects of NO on neutrophils include the inhibition of superoxide anion production and adhesion to endothelial cells (Clancy et al. 1992; Kubes et al. 1991; Stefanovic-Racic et al. 1993).

In response to various stimuli, activated inflammatory cells (e.g., eosinophils and neutrophils) generate oxidants (“oxidative stress”), which in conjunction with exaggerated enzymatic release of $\text{NO}^\cdot$ and augmented $\text{NO}^\cdot$ metabolites produce the formation of strong oxidizing reactive nitrogen species, such as peroxynitrite anions (ONOO^-).

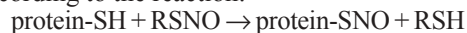


These peroxynitrite anions are involved in progression of various types of airway diseases such as asthma, chronic obstructive pulmonary diseases (COPD), cystic fibrosis and acute respiratory distress syndrome (ARDS). Reactive nitrogen species are involved in the amplification of inflammatory processes in the airways and lung parenchyma causing DNA damage, inhibition of mitochondrial respiration, protein dysfunction and cell damage (“nitrosative stress”). This results into alteration in respiratory homeostasis (such as bronchomotor tone and pulmonary surfactant activity) and the long-term persistence of “nitrosative stress” may contribute to the progressive deterioration of pulmonary functions leading to respiratory failure (Ricciardolo et al. 2006).

Nitric oxide readily binds certain transition metal ions. Many physiological effects of NO[•] are exerted as a result of its initial binding to Fe(II)-haem groups in the enzyme *guanylate cyclase*:



Nitric oxide reacts fast with many radicals, e.g. with tyrosyl radical while it is generally unreactive with most non-radical substances. If cellular responses to nitrosative stress are similar to responses for oxidative stress, protein S-nitrosothiols (protein-SNO) might occur according to the reaction:



Protein S-nitrosothiols have been detected in animal blood and involve S-nitrosoalbumin, nitrosohaemoglobin and the least stable S-nitrosocysteine. S-nitrosothiols assume bioactivity through their capacity to donate NO and may therefore serve as stable intermediaries (Clancy et al. 1994).

Both ROS and end products of its reaction with the biomolecules lead to the production of several different by-products, which directly or indirectly interact with DNA causing damage by altering its purine and pyrimidine bases. OH[•] interacts with guanine base of DNA to form 8-hydroxyguanosine, which undergoes keto-enol tautomerism and forms 8-OHdG or, 8-oxoguanosine. Mitochondrial DNA has been shown to be most effected by oxidative stress (about 16 folds more) as compared to nuclear DNA. High levels of 8-OHdG have been found in mitochondrial DNA (Shigenaga et al. 1989) as a consistently high amount of ROS is produced by the mitochondria itself. Their production coupled to the fact that no specific mitochondrial DNA repair mechanism has been found, specially for nucleotide excision repair makes its genome more susceptible. Secondly, due to the absence of histone proteins in the mitochondrial DNA makes the nucleotides and hydrogen bonds easily approachable for reactions with the hydroxyl anions (Valko et al. 2006). ROS can also directly cause single and double strand breaks in the mitochondrial DNA strands (Durham and Snow 2006).

ROS also attack several cell organelles along with DNAs such as cellular components involving polyunsaturated fatty acids residues of phospholipids (Esterbauer et al. 1991; Marnett, 1999). Fe²⁺ had been found to catalyse Fenton type reactions. The mechanism, proposed more

than two decades ago by Bucher et al. (1983), involves the formation of a Fe(II):Fe(III) complex (or a Fe(II)-O₂-Fe(III) species) with the maximum rates of lipid peroxidation at the ratio 1:1 of Fe(II):Fe(III) (Bucher et al. 1983). Bucher et al. also demonstrated that ADP-Fe(II) promoted the peroxidation of phospholipid liposomes, but only after a lag phase (Valko et al. 2006; Bucher et al. 1983). Catalase, superoxide dismutase and hydroxyl radical scavengers did not extend the lag phase or inhibit the subsequent rate of lipid peroxidation (Valko et al. 2006). The final products of lipid peroxidation are malondialdehyde (MDA) and 4-hydroxy-2-nonenal (HNE). MDA can further react with the DNA bases G, C, and A to form M₁G, M₁C and M₁A. Of these M₁G has been found to undergo transition to A and transversion to T in the major groove of DNAs, while in the minor grooves it forms N²-oxopropenyl-G, which "predicts" its mutagenic properties. Furthermore, several other DNA base conversions had been reported in response to ROS with analogue base pairs such as ethenodA, -dC and -dG (Fedtke et al. 1990; Saparbaev et al. 1995) and hydroxypropanodeoxyguanosines (HO-PdGs) which are derived from the reaction of the DNA with acrolein and crotonaldehyde generated by lipid peroxidation (Nath and Chung 1994).

Figure 1 summarizes the major cellular effects after induction of oxidative stress including lipid peroxidation, changes of the endogenous level of calcium ions and activation of gene expression.

INTERACTION OF ROS WITH TARGET MOLECULES

The interaction and reaction of reactive oxygen species with target molecules can lead to DNA damage, activation of signaling cascades and finally to cancer development. An example for this pathway leading to cancer development is given in Figure 2 for inhalable particles and fibres. Airborne particles, e.g. silica, and asbestos fibres are able to activate the Nuclear Factor κB (NF κB), NF κB-dependent gene expression, and the mitogen-activated protein kinases (MAPK), c-Jun-NH₂-terminal amino kinases (JNK), p38 kinases and extracellular signal-regulated kinases (ERKs) (Desaki et al. 2000; Jansen et al. 1995). Phosphorylation of various members of the MAPK cascade may result in activation of a number of transcription factors that interact with

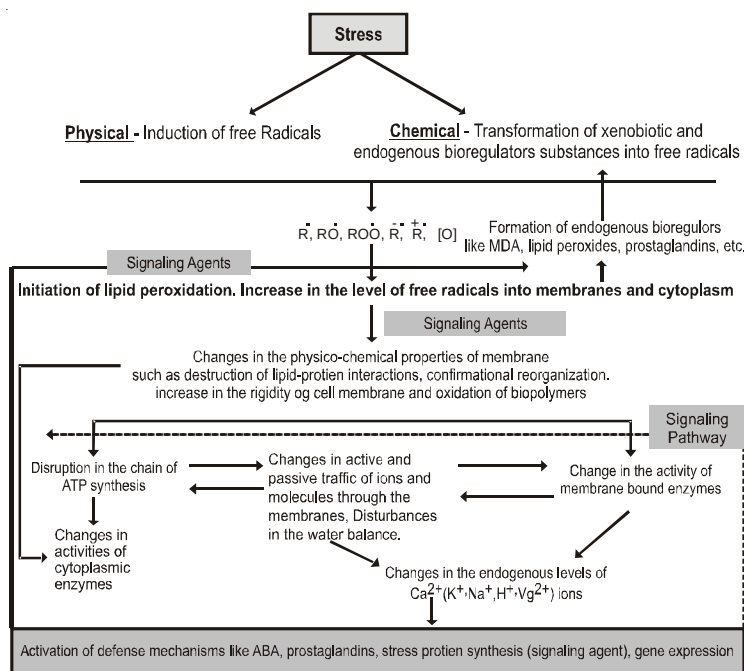


Fig. 1. Oxidative stress and cellular signalling pathways.

regulatory domains with the promotor region of genes that are linked to cellular responses such as differentiation, proliferation or apoptosis (Fig. 2). Initiation of proliferation in epithelial cells by particles/fibres may occur after upregulation of early response proto-oncogenes (c-fos, c-jun) (Timblin et al. 1995).

For an individual initiated cell to survive and not to evolve into an uncontrolled growing and proliferating cell, it is required to maintain the integrity of the DNA molecule before initiating DNA replication, transcription and cell division. Therefore, cells are provided with several efficient DNA repair mechanisms. Furthermore, the process of DNA damage itself triggers cell cycle arrest. This provides time for DNA repair and initiates signal transduction pathways involved in cell cycle control and apoptosis. Figure 3 shows the p53- and RB-dependent pathway of cell cycle regulation and its deregulation caused by asbestos. In relation to cell cycle regulation and apoptosis induction, the tumour suppressor protein p53 represents a major component studied in particle/fibre research to date. It is well known that DNA damage results in upregulation of p53, and this correlates with the induction of

apoptosis or cell cycle arrest (Fig. 3), although the specific conditions that determine the fate of either of these processes are still poorly understood. Increased p53 expression has for instance been observed in asbestos-exposed human lung epithelial cells (Matsuoka et al. 2003) and in human mesothelial cells (Burmeister et al. 2004). Mesothelioma induction by asbestos was found to be accelerated in heterozygous p53^{+/−} mice compared to wild-type animals (Vaslet et al. 2002).

Frequent genetic changes found in asbestos-induced human malignant mesothelioma (HMM) are deletions and point mutations in the tumor suppressor genes p16^{INK4a} and NF2. Homozygous deletions appear to be the predominant mechanism leading to p16/CDKN2A inactivation. Inactivating point mutations coupled with allelic loss mainly occur at the NF2 locus. A disarrangement of the RB1 dependent pathway in mesothelioma formation was suggested by Dopp et al. (2002). As the NF2 gene is frequently mutated in HMM, Lecomte et al. (2005) exposed hemizygous NF2 mice and their wild type counterparts to asbestos fibres. Higher rates of tumoural ascites and peritoneal tumours were observed in

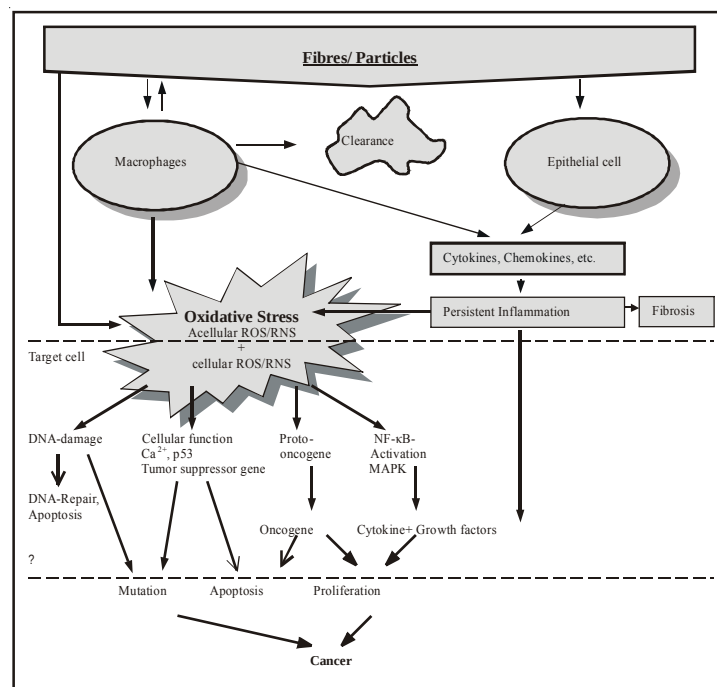


Fig. 2. Particle/fibre-induced interaction of reactive oxygen species with target molecules leading to cancer development

the hemizygous NF2 than in the wild type mice. The histological features of the tumours were similar to those of human HMM. Ascites fluid was cultured and biallelic inactivation of the NF2 gene was noted in 90% of primary cultures. From these results, it can be postulated that asbestos exposure may entail inactivation of tumor suppressor genes, and that a mutation on one allele prior to exposure would potentiate the carcinogenic effect. On the other hand, there is certain common molecular changes in tumor suppressor genes, which may be involved in the pathogenesis of HMM (Table 1).

Several research groups have also studied the effects of particles/fibres on the activation of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), in relation to its function as a biomarker of DNA damage and/or repair. PARP, which can be activated by DNA strand breaks, is involved in the ribosylation of DNA polymerase and topoisomerase I, and its prolonged activation is associated with cell death. Particles that have been shown to activate PARP include asbestos and silica (Tsurudome et al. 1999; Ollikainen et al. 2000; Kamp et al. 2001).

ROS-induced Genomic Damage Caused by Particles/Fibres

ROS/RNS can oxidatively attack DNA, leading to structural alterations in the DNA, such as base pair mutations, deletions or insertions, which are all commonly observed in mutated oncogenes and tumour suppressor genes. As such, it is obvious that persistent formation of oxidants, e.g., as occurring during particle/fibre-induced inflammation, is considered as a crucial factor in particle/fibre-related genotoxicity and carcinogenicity.

Excessive generation of ROS that overwhelms the antioxidant defense system can oxidize DNA and generate a large number of oxidative DNA modifications, including strand breaks and base oxidations. Among oxidative DNA damage products, 8-oxo-7,8-dihydro-22-deoxyguanosine (8-oxodG) is probably the most studied oxidation product due to its relative ease of measurement and pre-mutagenic potential (Kasai 1997). In DNA, 8-oxodG may be formed by oxidation of guanine or incorporated during replication or repair as oxidized nucleotides (8-

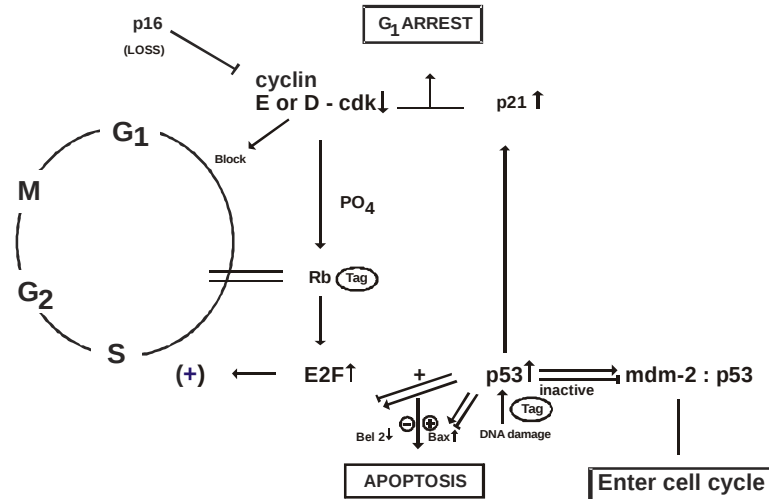


Fig. 3. The p53- and RB-dependent mechanisms of cell cycle regulation are influenced after exposure of cells to asbestos fibres. The asbestos-induced DNA damage during G1-phase increases the intracellular p53-level and activates p21-transcription. This activation blocks cdk-activity. The cell is arrested in G1-phase. Mdm-2 is a negative regulator for p53 and can release the cell from G1-arrest (together with increased expression of E2F). The process of apoptosis is regulated by inhibitors (e.g. Bcl-2) and activators (e.g. Bax). The SV40-Tag is able to inactivate mdm-2 and Rb.

Table 1: Common molecular changes in TSG involved in the pathogenesis of HMM (adapted from Jaurand et al. 2005)

Molecular changes	Cellular perturbation	Potential aetiological factor involved
<i>P16/CDKN2A</i> and <i>P15/CDKN2B</i> : frequent inactivation, mainly by deletion.	Cell cycle: loss of control of cell proliferation at the G1-S transition.	Asbestos: frequent inactivation of the murine homologue <i>p16/Cdkn2a</i> is observed in mesothelioma cells from asbestos-exposed mice. <i>P19/Arf</i> is also inactivated.
<i>NF2</i> : frequent inactivation.	Destabilization of adherens junctions. Loss of negative control of cell proliferation.	Asbestos: frequent loss of heterozygosity of <i>NF2</i> in mesothelioma cells from mice, hemizygous for <i>NF2</i> , exposed to asbestos fibres.
<i>TP53</i> : inactivation. a) low rate of point mutation b) binding to viral proteins.	Cell cycle control: inactivation of checkpoints controlling apoptosis and cell cycle progression after DNA damage.	a) Asbestos: low rate of point mutations in the murine homologue <i>Trp53</i> is found in mesothelioma cells from asbestos-exposed mice. Loss of heterozygosity in mesothelioma cells from mice, hemizygous at the <i>Trp53</i> locus, exposed to asbestos fibres. b) SV40: binding to large T antigen in human mesothelioma.

NF2, neurofibromatosis 2.

oxodGTP). Oxidative DNA damage is repaired by a number of different enzymes. This implies that, at the time of measurement, the level of oxidative DNA damage in intact cells or animal experimental

models exposed to particles must be interpreted as a steady-state level. DNA base lesions mainly are repaired by base excision repair enzymes. OGG1 is the base excision repair enzyme that is

involved in removal of 8-oxodG (Nishimura 2002). The MTH1 gene encodes an 8-oxodGTPase that hydrolyzes 8-oxodGTP. Its gene product is revealed by a larger number of tumours in MTH1-deficient mice (Tsuzuki et al. 2001). Other repair pathways for 8-oxodG include nucleotide excision repair processes, mismatch repair, and NEIL proteins (Endonuclease III) (Bjelland and Seeberg 2003).

In cell culture experiments, Diesel Exhaust Particles (DEP) caused strand breaks and 8-oxodG formation. The majority of studies have investigated DEP genotoxicity in A549 lung epithelial cells. However, fibroblasts, freshly isolated leukocytes, and cells with metabolic capabilities (HepG2 and Caco-2) have also been used as target cells. In general, aqueous and organic DEP suspensions (or particle free compounds extracted in water and organic solvents) induce DNA damage in all of the investigated cell types (Table 2). This suggests that both the particle and constituents of particles possess the ability to cause oxidative DNA damage.

Previous studies have shown that asbestos fibers (this is not known for particles) are able to modulate the ERK cascade (intracellular signaling pathway) in primary rat pleural mesothelial cells (Mossman et al. 1997). Stimulation of the ERK cascade has been shown to be involved in fiber-induced apoptosis in this cell type. Activation of ERK by asbestos in mesothelial cells is mediated through phosphorylation of the epidermal growth factor receptor (EGFR) via generation of reactive oxygen species (ROS), in as much as EGFR tyrosine kinase inhibitors and antioxidants such as *N*-acetyl-L-cysteine (NAC) are capable of blocking this response. The downstream effect of p38 MAPK activation on crocidolite-induced AP-1 DNA binding was investigated by Swain et al. (2004). The AP-1 transcription factor is extremely well understood, being composed of Jun: Jun homodimers or Jun: Fos heterodimers. The exact composition may influence which subset of genes is targeted for transcription by the complex. Given that there are multiple members of the Fos and Jun protein family, this may account for the pleiotropic effects of AP-1 activation.

Genes Which are Especially Susceptible to Oxidative Damage

Since the reduction and oxidation of

disulphide bonds occur at the posttranslational level, **p53** is considered as one of the oxidative stress response transcriptional factors (Renzing et al. 1996). The p53 tumor suppressor gene is located on the chromosome 17p13.1. The protein has several cysteine residues in the central domain, and these amino acid residues are important for the binding of p53 to specific DNA. Mutations of p53 have been found to be one of the common causes in several cancers including mesothelioma and lung cancer (Hollstein et al. 1991; Wasielewski et al. 2006). Also, production of ROS has been correlated with p53-mediated apoptosis (Polyak et al. 1997). In this regard, increased intracellular ROS induces the expression of two pro-apoptotic proteins (ferrodoxin reductase (FDXR) and REDD/HIF-1). In addition to these two proteins, p53 also induces the expression of p85, which may function as a signaling molecule during ROS-mediated p53-dependent apoptosis. Panduri et al. (2006) studied the inhibitors of p53-dependent transcriptional activation (pifithrin and type 16-E6 protein) and observed that they blocked asbestos-induced AEC mitochondrial membrane potential change, caspase 9 activation, and apoptosis. Asbestos enhanced p53 promoter activity, mRNA levels, protein expression, and Bax and p53 mitochondrial translocation. They further observed that asbestos augments p53 expression in cells at the bronchoalveolar duct junctions of rat lungs and that phytic acid prevented this. These studies suggest that p53-dependent transcription pathways mediate asbestos-induced AEC mitochondria-regulated apoptosis suggesting an important interactive effect between p53 and the mitochondria in the pathogenesis of asbestos-induced pulmonary toxicity including pulmonary fibrosis and lung cancer. Scian et al. (2005) observed that overexpression of mutant p53 was a common theme in several tumors, suggesting a selective pressure for p53 mutation in cancer development and progression. The authors generated stable cell lines expressing p53 mutants p53-R175H, -R273H, and -D281G by use of p53-null human H1299 (lung carcinoma) cells in order to determine how mutant p53 expression may lead to survival advantage in human cancer cells. Cells were treated with etoposide, a common chemotherapeutic agent and transactivation-deficient triple mutant p53-D281G (L22Q/W23S) cells were observed which had significantly lower resistance to etoposide. All the three types of

Table 2: Oxidative DNA damage induced by particulate matter in vitro (modified from Risom et al. 2005)

<i>Particle type (solvent)</i>	<i>Concentration (µg/ml)</i>	<i>Time (h)</i>	<i>Endpoint (assay)</i>
PM _{2.5} urban particles (aqua)	33	3	SB (comet)
SRM1650 (aqua)	10–500	3, 5, 24	SB (comet)
SRM1650 (aqua)	0.016–1.6	48	SB (comet)
Bentonite	5–15 µg/cm ²	24–72	micronuclei
Urban particles (Dusseldorf, Germany)	10–100	24	SB (FADU)
Urban particles (Leeds, UK) (aqua)	25	24	SB (comet)
SRM 1649 (aqua)	0.1–100 µg/cm ²	24	SB (comet)
PM ₁₀ subway or street particles (aqua)	5–40 µg/cm ² (comet) or 10 µg/cm ² (8-oxodG)	4	SB (comet) and 8-oxodG (HPLC-EC)
SRM1649	4 × 10 ⁵	2	8-oxodG (HPLC-EC)
Fine urban particles (aqua)	40, 200	3	SB (comet)
PM _{2.5} and PM ₁₀ urban particles (aqua)	50	2	8-OxodG (IHC)
PM ₁₀ urban particles (not specified)	5–20	72	SB (comet)
PM ₁₀ urban particles (dichloromethane)	5–150	2	SB (comet)
PM ₁₀ urban particles (dichloromethane)	1–50	24	SB ± ENDOIII/FPG (comet)
PM ₁₀ urban particles (aqua or tetrahydrofurane)	5–20 m ³ air equivalents/ml	24	SB (comet)
PM _{2.5} urban particles (dichloromethane)	0.8–2.1	72	SB (comet)
TSP, PM ₁₀ , PM _{2.5} (acetone or toluene)	1–3 m ³ air equivalents/ml	1	SB (comet)

transcriptionally active p53 mutants induced expression of approximately 100 genes involved in cell growth, survival, and adhesion with the NF-κB2 being the most prominent member of this group. Over expression of NF-κB2 in H1299 cells also leads to chemoresistance. Thus, one possible pathway through which mutants of p53 may induce loss of drug sensitivity and loss of apoptotic functions in the cancer cells might be through the activation of the NF-κB2 pathway. Another mechanism by which metals (especially iron) affect p53 is through zinc substitution, which is essential for the binding of p53 to DNA. Metals substituting zinc can inactivate p53 without mutation or oxidation. Studies had also confirmed mutations in the p53 gene following exposure to NO[•]. There is an evidence of G: C to A: T base pair transition following exposure to iNOS in the cells. Therefore, NO[•] and its derivatives can function as an internal initiator and promoter for human carcinogenesis.

HIF-1 (Hypoxia inducible factor-1) is a nuclear transcription factor, which mediates not only oxygen- but also iron regulated transcriptional gene expression (Martin et al. 2005). It is a

heterodimer composed of βHLH protein, HIF-1α and -1β (Du et al. 2004) and is located in human chromosome 3 at position 3q21.1. HIF-1 regulates the expression of several cancer-related genes including vascular endothelial growth factor, aldolase, enolase, lactate dehydrogenase A, etc and therefore plays an important role in solid tumor cell growth and survival (Sasabe et al. 2005). HIF-1α is a transcription factor involved in the cellular adaptation to either hypoxia or iron deficiency. In the presence of oxygen and iron, proline residues in two degradation domains are modified by HIF-1-prolyl hydroxylases (PHDs), resulting in ubiquitination and degradation of HIF-1-prolyl hydroxylasesubiquitination and degradation. Since both molecular oxygen and iron are required for the hydroxylation of proline, HIF-1α might be unmodified and stable in hypoxic as well as in iron deficiency conditions. It was found that the roles of the two degradation domains could be dissected based on experiments demonstrating that, the C-terminal domain was responsive to both hypoxia and iron-depletion, while the N-terminal domain responded to only iron-depletion

Table 2: Contd.....

<i>Cell or DNA type</i>	<i>Effect</i>	
Myeloid leukaemia (K562)	SB increased in both cell types. Probably no difference	
Lung epithelia cells (IB3-1)	between particle samples	Dellinger et al. 2001
Lung epithelial cell (A549)	SB increased 100 and 500 µg/ml after 2, 5, 24	Dybdahl et al. 2004
Lung epithelial cell (A549)	Increased SB at 0.016 µg/ml (THP-1) and 0.16 µg/ml (A549)	Don Porto et al. 2001
Monocyte (THP-1)		
Lung fibroblasts (IMR90)	weak genotoxic effects at 15 µg/cm ²	Geh et al. 2006
Lung epithelial cell (A549)	Increased SB at 10 µg/ml	Upadhyay et al. 2003
Lung epithelial cell (A549)	Increased SB (small size particles most potent)	Healey et al. 2005
Fibroblast (MUF)	Increased SB at 10 µg/cm ²	Karlsson et al. 2004
Lung epithelial cell (A549)	Increased SB at 10 (subway) and 20 (street) µg/cm ² . Increased 8-oxodG by subway, but not street particles	Karlsson et al. 2005
Airway epithelial cell (BEAS)	Increased level of 8-oxodG	Prahalad et al. 2001
Lung epithelial cell (A549)	SB increased at 200 µg/ml	Knaapen et al. 2002
Lung epithelial cell (A549)	Increased 8-oxodG in both preparations	Shi et al. 2003
Balb-c mouse fibroblast	SB increased at 5 µg/ml in two samples. Not increased in one sample	
Hepatoma (HepG2)	SB increased at 5 µg/ml	Alfaro-Moreno et al. 2002
Hepatoma (HepG2)	SB (±ENDOIII/FPG sites)	Gabelova et al. 2004
Colon carcinoma (Caco-2)	increased at 1 µg/ml	Lazarova and Slamenova, 2004
Leukocytes (±S9 mix)	SB increased at 10 (particle) or 20 (extracts) m ³ air equivalents/ml	Brits et al. 2004
Rat 6 rodent fibroblasts	Increased SB and difference in samples collected in different seasons	Hsiao et al. 2000
Leukocytes	Increased SB for all size fractions and extraction procedures	Buschini et al. 2001

signifying differential regulation of this transcription factor (Lee et al. 2006). The deletion or point mutation of the C-terminal domain blunted the hypoxic induction of HIF-1 α . PHD silencing via siRNAs revealed that two degradation domains were not regulated by different types of PHDs. It was found that both the domains were regulated mainly by PHD2. Further mutational analysis showed that ARD1-acetylated motif near the C-terminal degradation domain (CDD) modulated the oxygen-dependent regulation of HIF-1 α . Sasabe et al. (2005) examined HIF-1 α induced survival pathways in human oral squamous cell carcinoma cell (OSCC) lines. They found that forced expression of HIF-1 α suppressed hypoxia-induced apoptosis of OSCC lines by inhibiting cytochrome-c release from mitochondria, whereas, severe expression of HIF-1 α inhibited the generation of ROS, elevated intracellular Ca²⁺ concentration, reduced mitochondrial membrane potential, and led to the cytosolic accumulation of cytochrome c, resulting in the inactivation of caspase-9 and caspase-3. Anti-apoptotic Bcl-2 and Bcl-x (L) levels were increased and pro-apoptotic Bax and Bak levels were decreased in the HIF-1 α over-

expressing OSCC lines. Over-expression of HIF-1 α also increased the levels of phosphorylation of Akt and extra-cellular signal-regulated kinases (ERK). These findings indicated that HIF-1 α prevents apoptotic cell death through inhibition of cytochrome-c release and activation of Akt and ERK.

One of the most studied genes especially in the context of the metal particle and fibers induced carcinogenic growth is the **NF κ B**. It is an inducible and ubiquitously expressed transcriptional factor involved in the cell survival, differentiation, inflammation and growth (Pande and Ramos 2005). NF κ B is a DNA binding protein that interacts with the enhancing domain of target gene in configuration of a dimer of two members of the NF κ B/Rel/Dorsal (NRD) family of proteins. In un-stimulated cells, NF κ B is sequestered in the cytoplasm because of an interaction with the members of the inhibitory (I κ B) family. Activation of NF κ B occurs in response to a variety of extracellular stimuli that promote the dissociation of I κ B, which unmasks the nuclear localization sequence and thereby allows entry of NF κ B into nucleus and binds κ B

regulatory elements (Valko et al. 2006). Treatment of the T47D cells with hydrogen peroxide induced the appearance of a modified form of inhibitor $\text{I}\kappa\text{B}\alpha$, which is rapidly degraded upon appropriate stimulation unless proteolysis is inhibited by a proteasome inhibitor (Kretz-Remy et al. 1996). As $\text{NF}\kappa\text{B}$ is the gene that is involved in cellular transformation, proliferation and angiogenesis, it is linked to the carcinogenesis process (Amiri et al. 2005). ROS has been implicated in several studies to act as a second messenger and leads to the activation of the $\text{NF}\kappa\text{B}$ via Tumor Necrosis Factor ($\text{TNF-}\alpha$) and Interleukin-1 (Hughes et al. 2005; Baud and Karin 2001). While, it is also the first eukaryotic nuclear transcription factor that has been shown to be directly activated by oxidative stress in certain types of cells (Schreck and Baeuerle 1991), $\text{NF}\kappa\text{B}$ has been found to be activated even by a moderate shift towards pro-oxidative conditions in cells such as, Molt-4 and Jurkat T cell lines (Galter et al. 1994; Hehner et al. 2000). Carcinogens such as UV, toxic metals, fibers such as asbestos, etc. have been found to act as external stimuli for the activation of the $\text{NF}\kappa\text{B}$ (Knight 2000). The activation of the $\text{NF}\kappa\text{B}$ by metals and other ROS is further supported by the studies that had demonstrated that its activation could be blocked by the antioxidants such as L-cysteine, N-acetyl cysteine (NAC), thiols, green tea polyphenols and Vitamin E.

Similar to the $\text{NF}\kappa\text{B}$ gene, another gene **AP-1** is also activated upon stimuli from oxidative stress. AP-1 gene is involved in the expression of a dimeric basic region-leucine zipper protein that belongs to the Jun (c-Jun, JunB, JunD) family, which can bind to tumor-promoting agents (TPA) and cAMP response elements (Rao et al. 1997). AP-1 activity is induced in response to certain metals in the presence of H_2O_2 as well as several cytokines and other physical and chemical stresses. *In vitro* transcriptional activity of AP-1 is regulated by redox state of a specific cysteine64 located at the interface of its two Jun subunits (c-Jun and c-Fos), highlighting the importance of the redox status on gene transcription. However, *in vivo* experiments demonstrated the requirement of this cysteine64 is not required for activation of AP-1 (Pinkus et al. 1996). The activation of AP-1 in all the cases is mediated mainly by JNK and p38 MAP kinase cascades (Pinkus et al. 1996). It was found that oxidatively activated MAPK phosphorylates serine residues 63 and 73 of the NH_2 terminal transactivation domain of c-Jun, which is required

for functional activation (Karin 1995; Karin and Smal T 1992). AP-1 activation has been found to effect cellular proliferation and its role along with cellular oxidants in cancer progress is well documented (Hsu et al. 2000). Expression of c-Jun and c-Fos can be induced by a variety of compounds, involving reactive radicals, non-genotoxic and tumor promoting compounds (e.g. - metals, carbon tetrachloride, Phenobarbital, TPA, TCDD, alcohol, ionizing radiation, asbestos, etc.). The mRNAs of c-Fos and c-Jun are induced by relatively small amounts of H_2O_2 , superoxide, NO and other inducers of oxidative stress (Dröge 2002; Janssen et al. 1997; Morris 1995; Meyer et al. 1993). Additionally, AP-1 along with acting as a cellular proliferation agent, also acts as a positive or negative agent for apoptosis, which in turn is dependent on the balance between the pro- and anti-apoptotic genes, the stimulus used to activate AP-1 and also on the duration of the stimuli. Oxidative stress results in AP-1-mediated induction of IL-2. Exposure of Jurkat T lymphocytes and a murine T cell line (Esb) to $200\mu\text{M}$ of H_2O_2 in the absence of a mitogenic stimulus, resulting in an enhancement in the expression of c-Jun and c-Fos. But, in the former, there was a lack of induction of interleukin-2, while in the latter, expression of interleukin-2 was concomitant with AP-1 expression (Los et al. 1995; Beiqing et al. 1996). These proteins have also been seen to be actively involved in cellular transformation along with oncogenes such as Ha-ras (Valko et al. 2006).

Besides the nuclear transcription factor genes, several other genes are directly or indirectly associated with them in the process of apoptosis or in the antioxidant defense mechanism. Mutagenic changes in these genes had been discovered to play an important role in carcinogenic formations, by affecting the apoptotic pathway in one way or the other. Some of the interesting genes that have been implicated to cause carcinogenic effects, especially when they are activated as a response to mineral particles and fibers are described below.

The most studied gene locus that is most likely to get affected by the oxidative stress after exposure to particles and fibers are the **INK4a/ARF** locus encoding two physically linked tumor suppressor proteins, p16(INK4a) and ARF, which in turn regulate the RB and p53 pathways, respectively. Pulmonary adenoma progression 1 (Papg-1) was mapped to a region on mouse chromosome 4. This locus contains a candidate

gene, CDKN2a also referred to as INK4a/Arf, which dually encodes two established tumor suppressors p16(INK4a) and ARF (Zhang et al. 2002). The INK4a (MTS1, CDKN2) gene encodes an inhibitor (p16/INK4a) of the cyclin D-dependent kinases CDK4 and CDK6 that blocks them from phosphorylating the retinoblastoma protein (pRB) and prevents exit from the G₁ phase of the cell cycle (Fig. 3). Deletions and mutations involving INK4a occur frequently in cancers, implying that p16/INK4a, like pRB, suppresses tumor formation (Quelle et al. 1995). The locus of INK4a also has an overlapping region of p19/ARF, therefore, any deletion or mutation has a dual effect on both p16/INK4a and p19/ARF. Serrano et al. (1996) studied the INK4a deletion in the activated Ha-ras-transfected fibroblasts and observed that loss of the INK4a locus (deletion or mutation), can result in neoplastic transformation. They also reported studies on mice carrying a targeted deletion of the INK4a locus that eliminated both p16/INK4a and p19/ARF. These mice were viable and developed spontaneous tumors at an early age and were highly sensitive to carcinogenic treatments.

The ARF gene locus is closely associated with the INK4a gene. Any mutation in one affects the other one. The ARF is associated with the regulation of p53. Both p53 and its upstream regulator p14/ARF (p53 apoptosis pathway) has been observed to be affected in over 93% of non-small cell lung carcinoma (NSCLC). Furukawa et al. (2004) found that aberrant methylation of cytosines in CpG islands of the promoter regions of tumor suppressor genes like ARF is found in human tumors and was a common mechanism of gene silencing. Zemaitis et al. (2005) found methylation of several tumor suppressor genes including ARF and INK4a in the lung serum of lung cancer patients. From this study, it was observed that aggressive growth of lung cancers was mostly associated with the methylation of one or more tumor suppressor genes (Mori et al. 2004).

Cyclin D1 gene (**CCND1**) is a key regulator of the G₁ phase of the cell cycle whose deregulation has been seen to play a very important role in tumorigenesis (Huang et al. 2006). It is present on the chromosomal location 12q13. Cells such as the airway epithelial cells are at greater risk of DNA damage because of their constant exposure to particulate matter (PM) -induced oxidative stress and carcinogens (Risom et al. 2005; Clement

et al. 2001; Li and Nel 2006b). In the presence of DNA damage, a control mechanism that induces cell cycle arrest is activated to ensure the fidelity of DNA replication and genomic integrity (Clement et al. 2001). This control is exerted at the cell cycle checkpoint in the G₁ phase (Clement et al. 2001; O'Reilly 2005; Corroyer et al. 1996). At this phase, cells have to make the decision whether to complete another round of cell division or to exit cell cycle and arrest at the G₀ phase. Cell cycle progression is controlled by the sequential assembly of cyclins and cyclin-dependent kinases (CDK). In the absence of mitogens, cells remain quiescent in the G₀ phase, but they activate cyclin D1 expression in a ras-dependent fashion in the presence of mitogens. CDK is active as a serine/threonine kinase if it associates with a cyclin protein and becomes activated by phosphorylation and dephosphorylation through Cyclin-Activated Kinase (CAK), weel kinase and cdc25 phosphatase (Morgan 1997; Jackman and Pines 1997).

The promoter for the cyclin D1 gene contains multiple DNA binding sites for transcription factors that can be activated by growth factors to regulate its expression (Herber et al. 1994; Watanabe et al. 1998; Hunter 1997; Roussel 1998; Lukas et al. 1996; Altucci et al. 1996; Brown et al. 1998). Adhesion of the cells to the extracellular matrix also activates the cyclin D1 complex by MAPK pathway via the binding of the integrin to the extracellular matrix component (Assoian 1997; Bottazzi and Assoian 1997; St Croix et al. 1998; Schlaepfer et al. 1994). It has been showed that over-expression of the cyclin D1 accelerates the G₁ transition whereas its inactivation by microinjection of antibodies or antisense DNA construct induced a G₁ stage arrest (Quelle et al. 1993; Lukas et al. 1995; Arber et al. 1997). Activation, inactivation as well as over expression of cyclin D1 is dependent on the different genes including p53, RB, p21, p27. Any mutation or loss of control in one of these genes due to oxidative stress and mutation is capable of creating uncontrolled expression of cyclin D1 therefore, leading to unregulated cell division (Michalides 1999). Weinstein (2000) suggested an existence of a feedback loop between cyclin D1 or cyclin E and p27^{Kip1}, the purpose of which is to maintain a homeostatic balance between positive and negative regulators of the G₁-S transition in the cell cycle. The increased levels of p27^{Kip1} in cancer cells might protect these cells from

potentially toxic effects of increased expression of cyclin D1 and/or cyclin E. p27^{Kip1} can easily arrest cell cycle in two different stages, G1 (Craig et al. 1997 and Katayose et al. 1997) and G2 (Wang et al. 1997). It was seen that the G2 blockage occurred by the inhibition of the cdk2 and cdk1 kinase activity. Similar studies done with p21^{Waf1} revealed altogether no apoptotic effect, thus showing that different cyclin dependent kinase inhibitors act in different ways (Wang et al. 1997).

Another important gene is the Retinoblastoma (Rb) gene. The Rb gene was the first tumor suppressor identified through human genetic studies. The most significant achievement after almost twenty years since it was cloned is the revelation that Rb possesses functions of a transcription regulator. Rb serves as a transducer between the cell cycle machinery and promoter-specific transcription factors. It can be inactivated by phosphorylation by cyclin-dependent kinases, in particular cyclin D/Cdk4 (Jinno et al. 1999). Assay protocols that express wild type Rb in Rb mutant tumour cells have been used to study Rb function (Huang et al. 1988; Goodrich et al. 1991). Results from these assays suggest that Rb can inhibit cell cycle progression from G1 to S (Goodrich et al. 1991; Hinds et al. 1992). It was also clearly observed in the Rb knockout mice study that they entered S phase much faster than those having the wild RB type gene (Zhu 2005; Herrera et al. 1996). While somatic mutations in the RB gene are associated with almost all sporadic retinoblastomas and small cell lung cancers (SCLCs), mutation of Rb is much less common in other human cancers (DeGregori 2006). One of the major findings till date has been the close association between Rb protein and Ras oncogene-mediated tumorigenesis. Ras with its still functionally indistinguishable two isoforms, Harvey-Ras and Kirsten-Ras has been well established to be activated by oxidative stress, which, in turn, affects the MAPK pathway-mediated activation of several downstream effectors. This signal transduction cascade may result in transformation of normal cells (Cuda et al. 2002). It has been established that Ras-MAPK pathway leads to the growth factor-dependent upregulation of cyclin D-dependent kinase activity (Sherr 2002). The final target of this activated cyclin D:cdk4 or cdk6 kinase complex is the Rb (Weinberg 1995). Phosphorylation of this protein (Rb) releases E2F transcription factors which are required for the transcription of E2F

responsive genes, including those with proteins involved in DNA synthesis and mandatory for S phase progression (Weinberg 1995). E2F represents a family of at least five different transcription factors, which bind to hypophosphorylated Rb-gene (Beijersbergen et al. 1995). pRb appears unique among these E2F binding proteins, since elimination of pRb alone is sufficient to liberate abundant E2F activity to render growth of cells growth factor independent (Herrera et al. 1996). Once activated, each of these E2Fs acts as a transactivator and mediates transcription of E2F responsive genes, among which are cyclins D1, E, and A. The promoters of cyclins D1, E, and A contain E2F binding sites (Watanabe et al. 1998; Muller et al. 1994).

It has been observed that mutational inactivation of the RB gene in a cell leads to increased expression of p107, p16INK4a, and ARF, which prevents Ras-dependent transformation of the normal cells. While, inactivation of p16 leads to higher G1 CDK activity, resulting in reduced function of these three Rb family proteins without ARF upregulation in turn promoting Ras-dependent transformation (DeGregori 2006) (Fig. 4). Dopp et al. (2002) studied human malignant mesothelioma and found that its occurrence can be correlated to

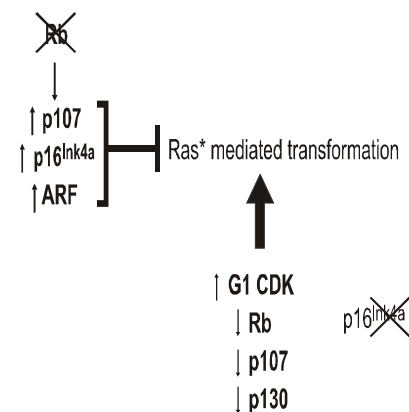


Fig. 4. Differential effects of Rb mutation versus G1 CDK deregulation on Ras-dependent transformation. Mutational inactivation of the Rb gene in a cell leads to increased expression of p107, p16INK4a, and ARF, which prevents Ras-dependent transformation. In contrast, inactivation of p16 leads to higher G1 CDK activity, resulting in reduced function (i.e., increased phosphorylation) of all three Rb family members without ARF upregulation, which promotes Ras-dependent transformation. (taken from DeGregori 2006)

hemizygous loss of one allele of RB1 gene thus disrupting its normal pathway of cell cycle in normal human mesothelial cell on exposure to asbestos.

P21 ^{Waf1/Cip1/Sdi1} is an important gene involved in the cellular senescence/ cell growth arrest. It was observed to be activated in several cells undergoing senescence. The p21 gene product has been found to influence the cell cycle at both G1 and G2 stages depending upon the status of the RB protein. P21 is an inducible protein (el-Deiry et al. 1993) and thus is an important part of p53-induced cell cycle arrest (Waldman et al. 1995, 1996). It has been observed that in p21 null mice, p53 by itself was not able to arrest cell proliferation after DNA damage (Brugarolas et al. 1995; Deng et al. 1995). The p21 gene has been found to be overexpressed or mutated in several tumors (Vainio 2001) and its product is a member of cell cycle inhibitors that include p27 and p57 (Sherr and Roberts 1995) and can inhibit cyclin-dependent kinases (Harper et al. 1993). Elevated serum level of p21 was found in seven out of 21 patients of pneumoconiosis who developed cancer later on, including five lung cancers (Brandt-Rauf et al. 1992). This protein is also capable of blocking the DNA replication by binding to the proliferating cell nuclear antigen (PCNA) (Waga et al. 1994). Noda et al. (1994) observed p21 expression in the fibroblasts undergoing senescence after prolonged passage. Moreover, p21 has been observed to cause senescence in a p53-independent manner (Fang et al. 1999; Wang et al. 1999). One of the mechanisms observed to be used by the p21 for causing senescence is by accumulating ROS inside the cell, thus increasing its level (Macip et al. 2002). As it was observed over-expression of ROS within the cells lead to its growth arrest / senescence (Hagen et al. 1997; Caldini et al. 1998). Chen et al. (2000) exposed human primary lung fibroblast, IMR90 to different concentrations of H₂O₂ and observed significant expression of p21 genes in growth arrested cells. However, no p21 expression was observed in the cells undergoing apoptosis. The expression of p21 by induction of ROS within the cells is well recorded (Odom et al. 1992), but the proper mechanism for this is yet to be discovered; however one of the probable mechanisms can be via p53. Thus, equal to the role of p53, the p21's role in cellular senescence/ death along with its overexpression in the lung fibroblast and other cells leading to the

accumulation of ROS within the cells and formation of lung cancer needs an indepth study.

OXIDATIVE STRESS RESPONSE GENES. ROLE OF NRF2-MEDIATED SIGNALING PATHWAY

The deleterious effects of ROS may be counteracted by several in-built defence mechanisms in the cells. These mechanisms include the co-ordinated induction of specific genes that encode phase II detoxifying enzymes and oxidative stress-inducible proteins (Li and Nel 2006a; Holtzclaw et al. 2004) including NAD(P)H quinone oxidoreductase (NQO1), glutathione S-transferase P1 (GSTP), ferritin, metallothionein and peroxiredoxin 2 (Dommels et al. 2003) presenting targets for chemotherapy/ chemoprevention (Wasserman and Fahl 1997).

The induction of detoxifying and antioxidant enzymes by particulate matter in response to oxidative stress is regulated by the transcription factor Nrf2 interacting with the antioxidant response element (ARE) (Li and Nel 2006a). The ARE is a *cis*-acting enhancer found in the 5'-regulatory region of phase II enzymes and oxidative stress proteins (Wasserman and Fahl 1997). It is also known as the electrophile responsive element (EpRE) because of its activation by electrophilic compounds and it regulates genes at the transcriptional level (Holtzclaw et al. 2004). There are several factors that interact with EpRE: (1) primary factors that recognize specific DNA motifs, such as basic leucine zipper (bZip) proteins like Nrf2, Maf, Fos and Jun, (2) secondary proteins that are recruited to promoters by protein-protein interactions with the DNA-binding proteins; these molecules serve as transcriptional coactivators or corepressors; they are named p160 coactivators such as SRC, p/CIP, TIF-2, ARE-BP1, (3) tertiary proteins that interact with secondary proteins and are essential for induction of transcription by transcription factors, for example CBP and p300, (4) proteins that alter the architecture of chromatin (Zhu and Fahl 2001).

NF-E2 related factor 2 (Nrf2) mediates transcriptional activation of EpRE-dependent genes and is regulated post-transcriptionally. Under normal conditions, Nrf2 is bound to Kelch-like ECH-associated protein 1 (Keap1) that functions to keep Nrf2 in the cytoplasm. Keap1 is bound to the actin cytoskeleton through its Kelch domain and contains highly reactive

cysteine residues in its intervening domain. Inducers can react with these thiols, which leads to a conformational change in Keap1 and subsequently to the release of Nrf2. Release of Nrf2 can also be triggered by post-translational modifications of Nrf2. The question of how the inducer signal might trigger Nrf2 phosphorylation remains unresolved (Holtzclaw et al. 2004). However, a model has been described in which Nrf2 is phosphorylated by mitogen activated protein kinases (MAPKs) including ERK, JNK and p38. MAPKs in the JNK and p38 pathway are activated by many environmental stress stimuli and result in apoptosis. Once activated, these MAPKs can phosphorylate transcription factors such as Nrf2. Oxidative stress induced expression of MAPKs like Raf1, MEKK1, ASK1 and TAK1 enhance Nrf2 transcriptional activity (Owour and Kong 2002).

Keap1 plays an important role in the homeostatic rate of proteosomal degradation of the transcription factor. Keap1 represses Nrf2 transactivation not only by sequestering it into the cytoplasm, but also by facilitating its degradation by the proteasome pathway (Holtzclaw et al. 2004).

Free Nrf2 translocates to the nucleus, where it heterodimerizes with Maf through a basic leucine repeat region and this complex binds to the EpRE motif (Holtzclaw et al. 2004). This bound complex recruits a p160 coactivator, an antioxidant response element binding protein 1 (ARE-BP1) and CREB binding protein (CBP) or p300 (Zhu and Fahl, 2001). It interacts with Nrf2 and probably serves as a link with the basal transcriptional machinery to enhance transcription of the genes that encode phase II enzymes (Holtzclaw et al. 2004).

NQO1 is a phase II detoxifying enzyme that reduces substrates like quinones to hydroquinones, and its role in the protection against oxidative stress and neoplasia is established involving more than two dozen genes in response to electrophilic and oxidative stress. The NQO1 gene is induced in response to antioxidants and oxidants (Jaiswal 2000). The enzyme is expressed in many tissues requiring a high level of antioxidant protection. Two distinct regulatory elements of the NQO1 gene are the EpRE and the xenobiotic response element (XRE).

Elemental iron is required for cell growth and proliferation. Excess iron is harmful, because it can catalyze the formation of ROS via a Fenton reaction. For this reason, cells have mechanisms for the control of intracellular iron levels. Important among these is the iron storage protein ferritin (Tsui et al. 2000). Ferritin is a large multimeric protein composed of 24 subunits. There are two types of subunits, H and L. The H subunit of ferritin has ferroxidase activity and can convert Fe^{2+} to Fe^{3+} , whereas the L subunit is responsible for iron nucleation and protein stabilization (Pietsch et al. 2003). The H-to-L ratio within ferritin varies among tissues and is altered in certain diseases, for example in inflammation and cancer (Tsui et al. 2000). Ferritin sequesters free iron and in this way it minimizes the damage caused by oxidative stress. The ferritin shell of subunits is capable of sequestering approximately 4500 iron atoms (Tsui et al. 2000).

The regulation of ferritin in response to oxidative stress occurs via transcriptional control mediated by two linked EpREs (Fig. 5). In which the basal enhancer element (FER1) is embedded (Wilkinson et al. 2003). It is composed of an AP1-like motif and an SP1-like dyad region.

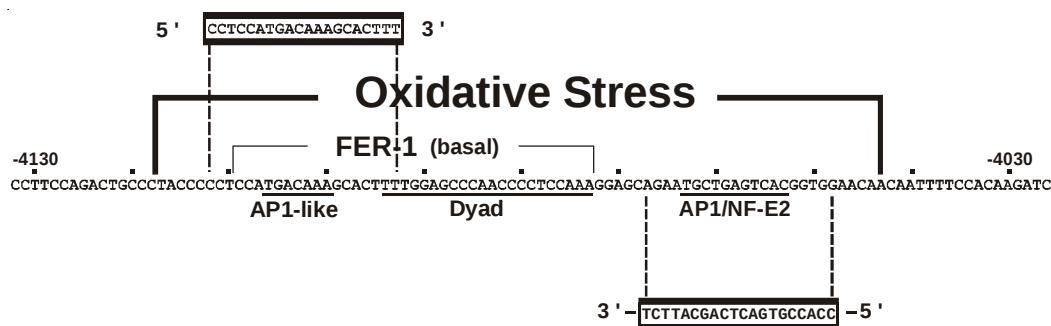


Fig. 5. Transcriptional regulation of ferritin (Tsui et al, 2000).

The AP1-like and the proximal AP1/NF-E2 consensus sequence are arranged in inverse repeat and both elements are required for full activity (Tsjui et al. 2000). Nrf2 is the most important factor that interacts with the AP1-like motif and AP1/NF-E2 consensus sequence (Pietsch et al. 2003) and the oxidative stress – mediated activation of ferritin H and L involving EpRE.

Ferritin mRNA is subject to translational control by iron regulatory proteins (IRPs). These proteins bind to the iron responsive element (IRE) in the 5' untranslated region (UTR) of ferritin mRNA. In this way translation of ferritin mRNA is inhibited. IRP1 activity is modulated by intracellular iron concentrations. At low iron levels IRP1 acts as a transcriptional repressor, at high concentrations IRP1 is inactivated. The activity of IRP1 is modulated not only by intracellular iron concentration but also by ROS. IRP binding to the IRE was stimulated rapidly due to exposure to hydrogen peroxide (Tsjui et al. 2000). Lipid peroxidation induces oxidative stress (Dotan et al. 2004) thus it can be possible that lipid peroxidation induces the activity of ferritin.

Metallothioneins (MTs) are the most abundant intracellular metal binding proteins. Their transcription is induced not only by oxidative stress, but also due to heavy metals such as cadmium and zinc. MT is an efficient scavenger of hydroxyl radicals and regulates cadmium and zinc homeostasis. Like ferritin, the MT promoter also contains EpRE (Fig. 6) (Andrews 2000).

The EpRE is negatively regulated by bZip proteins Fos and Fra1, and positively regulated by Nrf2 small Maf heterodimers in response to electrophilic agents. The EpRE overlaps an upstream stimulatory factor-binding site (USF), a member of the basic-helix-loop-helix Zip protein superfamily. The USF/ARE enhances basal level transcription of the mouse *MT1* gene. Furthermore metal responsive elements (MRE) respond to cadmium and zinc but also to oxidative stress. One study suggests that activation of *MT* gene

expression by oxidative stress is mediated by an increase in free zinc in the cell. This zinc acts than as a second messenger to activate the binding activity of the zinc finger transcription factor MTF1. MT can exert anti-oxidant activities but it is not known whether this is an essential function (Andrews 2000).

SUMMARY AND CONCLUSIONS

The overall epidemiological evidence is consistent with the hypothesis that exposure to airborne particles and fibers is an important risk factor in several lung diseases including cancer. The biological linkages are not fully understood, although the research to date, point to an involvement of oxidative stress and inflammation. Airborne particles and fibres contain redox-active substances and transition metals, which generate reactive oxygen species (ROS). These ROS may alter the balance between gene expression of pro-inflammatory mediators and antioxidant enzymes and therefore contribute to cancer development. Metallo-protein complexes like ferritin (binds Fe^{2+}) and metallothioneins (binds Cd, Cu, Zn and others) regulate the free metal content within the cell and control the main cause of metal-induced oxidative stress. A dysregulation of these complexes also leads to an increased intracellular radical formation.

The most sensitive cellular response to oxidative stress caused by particles/fibers is the activation of antioxidant and phase II enzymes. If this protection fails, further increase of oxidative stress can induce inflammation and cell death. The expression of these antioxidant enzymes is regulated by the transcription factor Nrf2, which interacts with the antioxidant response element (ARE) in the promoters of phase II enzyme genes, leading to their transcriptional activation (Li and Nel 2006a). Excessive generation of ROS that overwhelms the antioxidant defense system can oxidize DNA and generate a large number of oxidative DNA

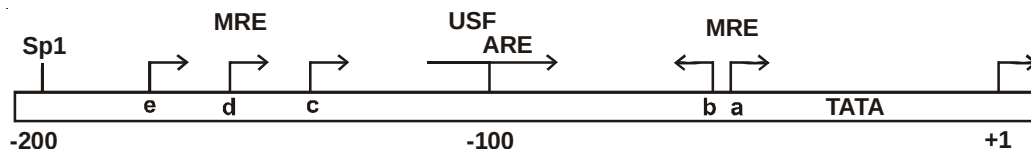


Fig. 6. Schematic representation of the mouse *MT1* promotor (Andrews, 2000).

modifications, including strand breaks and base oxidations. Among oxidative DNA damage products, 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) is probably the most studied oxidation product in particle/fibre research (Kasai 1997).

The effects of particles/fibres on the activation of the DNA repair enzyme poly(ADP-ribose) polymerase (PARP), in relation to its function as a biomarker of DNA damage and/or repair was studied by several groups. PARP, which can be activated by DNA strand breaks, is involved in the ribosylation of DNA polymerase and topoisomerase I, and its prolonged activation is associated with cell death. Particles that have been shown to activate PARP include asbestos and silica (Tsurudome et al. 1999; Ollikainen et al. 2000; Kamp et al. 2001).

In relation to cell cycle regulation and apoptosis induction, the tumour suppressor protein p53 represents a major component studied in particle/fibre toxicology to date. It is well known that particle/fibre-induced DNA damage results in upregulation of p53 (Soberanes et al. 2006, Matsuoka et al. 2003).

Recent data have also indicated that oxidative stress and pro-inflammatory mediators can alter nuclear histone acetylation/deacetylation allowing access for transcription factors to DNA binding leading to enhanced pro-inflammatory gene expression in various lung cells (Rahman 2002).

Altogether, analysis of gene expression profiles can play an important role in the early detection of particle/fibre-induced genomic damage that may lead to the development of severe diseases and malignancies. Monitoring expression of genes, which are important in pro-oxidative stress signalling, can play a major role in the regulation of transcription of several other genes, which for instance regulate expression of inflammatory cytokines. More research is needed especially in the field of particle-induced genomic alterations.

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