We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



4

Molecular Markers Associated with the Biological Response to Aromatic Hydrocarbons from Urban Air in Humans

Francisco Arenas-Huertero[1,2], Elisa Apátiga-Vega[2], Gabriela Miguel-Pérez[2], David Villeda-Cuevas[2] and Jimena Trillo-Tinoco[3]
[1]*Hospital Infantil de México Federico Gómez,*
[2]*Universidad Simón Bolívar,*
[3]*Hospital General de México*
México

1. Introduction

Morbidity and mortality attributable to air pollution continue to be a growing problem in several parts of the world. Both epidemiologic and clinical studies have demonstrated a strong link between exposure to particulate matter (PM) and adverse effects on health. From the PM generated in the atmospheres of several countries, the respirable fraction of $PM_{2.5}$ ($PM_{2.5}$) and diesel exhaust particles (DEP) represent some of the largest products of vehicular- and industrial-emitted airborne PM that can persist in the air, where they are readily inhaled and deposited throughout the respiratory tract. $PM_{2.5}$ and DEP have been associated with cardiac and pulmonary alterations. Also, exposure to DEP has been associated with lung cancer, pulmonary inflammation, an increased susceptibility to respiratory infections and the exacerbation of asthma and chronic obstructive pulmonary diseases. Furthermore, the effect of tobacco and its smoke, a complex mixture, represents another source of polycyclic aromatic hydrocarbons (PAH). The three, $PM_{2.5}$, DEP and tobacco smoke, are the main foci of several studies that evaluate the principal effects of PAH, and represent the via to/source of PAH exposure. Normally, black carbon particles, also products of incomplete fuel combustion, act as condensation nuclei for organic chemicals, such as aromatic aliphatic compounds, including PAH, but they are not considered in this chapter. Rather, this chapter will centre on a molecular description of the cellular responses (AHR pathway) after exposure to PAH from urban air, including relatively new markers, microRNAs and their utility as new biomarkers of exposure to PAH. We propose the use of lung tissue embedded in paraffin as a source of biological material to perform any kind of study: retrospective and prospectives. First, however, an important general description of PAH, its main sources and its concentrations in urban air and their metabolism will be presented in order to contextualize the main objective of this study of molecular markers associated with PAH exposure.

2. Polycyclic aromatic hydrocarbons (PAH)

Polycyclic aromatic hydrocarbons (PAH) are a complex class of condensed multi-cyclic of benzoic ring compounds, fused in linear, angular, or cluster arrangements that sometimes include a five-member ring, containing only carbon and hydrogen atoms. They have a basic structure and substituent moieties such as the alkyl, amino, chloro, cyano, hydroxyl, or thiol groups, and/or contain atoms such as nitrogen, oxygen, or sulphur in their aromatic structure. The most well known PAH is benzo[a]pyrene (BaP) (Gachanja, 2009; Wang et al., 2010).

The physico-chemical properties of PAH largely determine their environmental behaviour. They are semi-volatile substances under atmospheric conditions and frequently occur both in the vapour phase and attached to particles, depending on the vapour pressure of each PAH. Low molecular weight PAH, containing two or three fused rings, are more water soluble and volatile, and are found predominantly in the vapour phase. High molecular weight PAH containing more than three fused rings, which are primarily associated with particles, are found mainly absorbed in PM (Rajput et al., 2010). Generally, between 80% and almost 100% of PAH with 5 rings or more (which are predominantly particle-bound in the atmosphere) can be found associated with particles with an aerodynamic diameter of less than 2.5 μm, $PM_{2.5}$.The physico-chemical properties of PAH vary considerably, their semi-volatility makes them highly mobile throughout the environment; their deposition and re-volatilisation distribute them among air, soil and water bodies and most can be photo-oxidized and degraded to simpler substances (Office for Official Publications of the European Communities, 2001).

PAH are ubiquitous in the environment and are suspected or known mutagenic and carcinogenic agents. The United States Environment Protection Agency has listed 16 PAH as priority pollutants. These are: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[*a*]anthracene, chrysene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-cd]pyrene, dibenzo-[*a,h*]anthracene, and benzo[*ghi*]perylene (United States Environment Protection Agency [USEPA], 1993).

2.1 Main sources of PAH

PAH are produced in all processes of incomplete combustion of organic substances. Their production is favoured by an oxygen-deficient flame, temperatures in the range of 650-900°C and fuels that are not highly oxidized (Maliszewska-Kordybach, 1999). At the temperature of pyrolisis (about 700°C), aromatic ring systems are the most stable among the structural types present, while aliphatic C-C bond and C-H bonds readily break down to yield molecular fragments of a free radical character which then undergo recombination (Rajput & Lakhani, 2010).

Natural sources of PAH such as volcanic activity and forest fires do not significantly contribute to present-to-overall PAH emissions. Anthropogenic sources can be divided into two categories: the combustion of materials for energy supplies *(e.g.* coal, oil, gas, wood, biodiesel, *etc.)*; and combustion for waste elimination *(e.g.* incineration). The first category includes stationary sources that are considered the main producers of PAH in outdoor air, such as industry (mainly coke and carbon production, petroleum processing, aluminium sintering, etc.), refineries, residential heating (furnaces, fireplaces and stoves, gas and oil

burners), power and heat generation (coal, oil, wood and peat power plants), and mobile sources like cars, lorries, trains, airplanes and sea traffic (gasoline and diesel engines). The second category includes the incineration of municipal and industrial wastes. Other miscellaneous sources are unregulated fires, such as agricultural burning, recreational fires, crematoria, cigarette smoking, as well as volatilisation from soils, vegetation and other surfaces (Maliszewska-Kordybach, 1999).

2.2 Formation of polycyclic aromatic hydrocarbons from tobacco

There are two generally accepted mechanisms in the literature; the first involves the pyrogenesis of PAH by the thermal degradation of organic tobacco components into small reactive molecules and/or free radicals during the high temperature pyrolysis processes, followed by recombination reactions of these reactive species to yield PAH. The second mechanism involves the unimolecular cyclization, dehydration, aromatization and ring growth of high molecular weight tobacco components, such as phytosterols, long-chained saturated and unsaturated hydrocarbons, alcohols and esters. It has been suggested that both mechanisms exist during the high temperature pyrolysis/combustion of tobacco. Of the many individual constituent classes of tobacco (alkaloids, reductor sugars, polysaccharides, long-chain hydrocarbon waxes, amino acids, proteins, *etc.*), lipophilic components, such as phytosterols, saturated aliphatic hydrocarbons, and terpenoid compounds were believed to be the major precursors of PAH formed from a burning cigarette. There is no doubt that the pyrolysis of tobacco, tobacco extracts, and individual components of tobacco at temperatures greater than 700°C leads to the formation of PAH (McGrath et al., 2006).

Cigarette smoking and environmental tobacco smoke are other sources of air exposure. Smoking one cigarette can yield an intake of 20-40 ng of BaP. Smoking one pack of unfiltered cigarettes per day yields 0.7 μg/day of BaP, while smoking a pack of filtered cigarettes per day yields 0.4 μg/day (Agency for Toxic Substances and Disease Registry´s [ASTDR], 1995).

2.3 PAH in urban air

PAH concentrations in air can vary from less than 5 to 200,000 nanograms/cubic meter (ng/m^3). Although environmental air levels are lower than those associated with specific occupational exposures, they are a public health concern when spread over large urban populations. The background levels of 17 of the Agency for Toxic Substances and Disease Registry's (ATSDR) toxicological profile priority PAH in ambient air are reported to be 0.02-1.2 ng/m^3 in rural areas and 0.15-19.3 ng/m^3 in urban zones [ATSDR, 1995].

The results for 37 countries were compared with other PAH emission inventories. It was estimated that the total global atmospheric emission of these 16 PAH in 2004 was 520 gigagrams per year (Gg y_1) with biofuel (56.7%), wildfires (17.0%) and consumer product usage (6.9%) being the major sources; China (114 Gg y_1), India (90 Gg y_1) and United States (32 Gg y_1) were the three countries with the highest PAH emissions (Zhang & Tao, 2008).

Levels of individual PAH are monitored in outdoor air, but are always present with other PAH as part of a complex mixture. Levels of PAH in outdoor air are much lower than those encountered in occupational settings and there is no convincing evidence, as yet, to suggest that PAH in outdoor air are a significant cause of lung cancer in the general population.

Estimating the health effects due to PAH in outdoor air is made difficult by cigarette smoking, which is a source of PAH in itself, and which can affect outdoor sources in population studies. Therefore, people are always exposed to a number of different PAH, rather than single PAH on their own (Comittee on the Medical Effects of Air Pollutants [COMEAP], 2011). Furthermore, a specific PAH can be characteristic of the air in each country. For example, in Mexico City, benzo[*ghi*]pyrelene and benzo[*123cd*]pyrelene are the most representative in the atmosphere with annual medians from 1.119 ng/m^3 in the southwest zone, to 1.84 ng/m^3 in the central zone, where an important number of automobiles circulate because it is the downtown area. Otherwise, BaP was present from 0.265 ng/m^3 in the southwest zone to 0.455 ng/m^3 in the downtown area of Mexico City. In these same zones, total heavy PAH were 6.089 ng/m^3 in the downtown zone and 3.402 in the southwest sector (Amador-Muñoz et al., 2011). These measurements were obtained from the total PAH contained in $PM_{2.5}$. Again, this example reveals how important it is to take into account each geographical region with its characteristic PAH, which are significant in evaluating health risks.

3. Metabolism of PAH: background

Despite the fact that the human body has evolved inducible enzymatic detoxification and DNA repair systems over millions of years for efficient protection against natural toxic non-polar exogenous chemicals, given the tremendous amount and diversity of chemical pollutants that have recently permeated the environment, these systems may be saturated by excess toxicants without being fully adapted for a complete detoxification of all man-made molecules. Because the organism can not fully metabolize and inactivate all non-polar exogenous chemicals, this would explain why lipophilic carcinogenic environmental pollutants such as PAH can bio-accumulate in the adipose tissue and be toxic (Irigaray & Belpomme, 2010). In this way, mammals and many lower organisms metabolize PAH primarily by enzymatic oxygenation into epoxides, phenols, dihydrodiols, quinones, and water-soluble conjugates, in an attempt to make them more soluble and thus facilitate their excretion from the organism. This converts the pre-carcinogen into the ultimate carcinogen that covalently binds mainly to DNA, forming the DNA-adducts, and leads to carcinogenesis, v.g. lung cancer. The biological activity of PAH is dependent upon the molecular structure and their isomers show diverse carcinogenic activity (Gerhard, 2005).

In most cases, oxidation of PAH by cytochrome P450 (CYP) enzymes is an initial step of the activation process to produce the polar biochemically reactive electrophilic species (ultimate carcinogenic metabolites), capable of interacting with cellular macromolecules, particularly nucleic acids, as mentioned above, and proteins (Nebert et al., 2004; Weiling & Warshawsky, 2005).

Since the 2-4-ring PAH is poor enzyme inducer, it appears that the PAH-metabolizing pathways are mainly induced by BaP-type minor constituents. Gene-environmental interactions that magnify the polymorphic variability in the pulmonary bioactivation/detoxification capacity probably play a key role in individual susceptibility to or protection against, chemically-induced lung cancer. Hence, human exposure to PAH mixtures with a high content of BaP-type hydrocarbons confers a potentially higher health risk than PAH mixtures with a low content of pro-carcinogens (Elovaara et al., 2006).

3.1 Metabolism of PAH in liver

PAH are metabolized into various products by xenobiotic (drug)-metabolizing enzymes such as CYPs, epoxide hydrolase (EH), glutation transferase (GST), UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), NAD(P)H-quinone oxidoreductase 1 (NQO1), and aldoketoredustases (AKR). As was mentioned above, CYP play key roles in the initial step of oxidation of PAH. First, BaP is converted into radical cations through a one-electron oxidation mechanism by peroxidase activity and the resultant products can react with DNA to form unstable depurinating adducts. Second, AKR has been shown to convert B[a]p-7,8-diol to reactive B[a]P o-quinone via B[a]P-catechols. The reactive B[a]P o-quinone is able to interact with DNA, forming stable and depurinating DNA adducts. As mentioned: PAH can be activated by two enzymatic actions; one forms PAH diol-epoxides by CYPs, while the other produces PAH-*o*-quinones by AKR. Finally, it is suggested that the quinines formed are carcinogenic metabolites. These quinine derivatives are detoxified by NQO1. These reactive metabolites of PAH are further converted by so-called phase II enzymes such as EH, UGT, GST, SULT, NQO1 and AKR into more polar and detoxified metabolites. The EH catalyzes the hydrolysis of various oxides of numerous endobiotic and xenobiotic chemicals into less reactive and more polar dihyrodiols. GST is constitutively expressed in various human tissues. Cytosolic GST encoded by polymorphic members of the alpha, mu, pi and theta gene families and play important roles in the metabolism of a variety of toxic and carcinogenic compounds. Most of the glutathione conjugates are less toxic and more polar and can be excreted from the body. Glucuronidation is a major pathway for the detoxification of numerous carcinogens such as PAH and aryl and heterocyclic amines. The UGT superfamily consists of two families, UGT1 and UGT2. These enzymes convert the PAH intermediates into molecular forms that are more likely to be excreted from the organism. Sulfonation performed by SULT is generally thought to be a detoxification process. In humans, SULT consists of three families. However, sometimes SULT can activate certain promutagens to highly reactive sulphate esters that bind covalently to DNA. NQO1 catalyzes two-electron reduction of a wide variety of substrates, including the PAH o-quinone, into inactive products, such as PAH-hydroquinones. NQO1 has been shown to convert benzene-derived quinines into more inactive hydroquinones, thus protecting benzene-induced hematotoxicity. Finally, AKR converts PAH trans-dihydrodiols into reactive PAH o-quinones that form stable and depurinating DNA adducts. Thus, PAH-diols are shown to be activated by two enzymatic actions (Shimada, 2006; Shimada & Fujii-Kuriyama, 2004).

Human CYP1A2 is notable among family 1 enzymes for its capacity to *N*-oxidize arylamines, the major metabolic process involved in the bioactivation of arylamines to potent mutagenic or carcinogenic compounds. CYP1A2 is the principal family 1 enzyme expressed in the human liver, and CYP1A2 contributes significantly to the hepatic metabolism of drugs. Among liver CYP drug-metabolizing enzymes, CYP1A2 plays a predominant role in the metabolic elimination of caffeine and melatonin, as well as commercial drugs such as flutamide, lidocaine, olanzapine, tacrine, theophylline, triamterene, and zolmitriptan. This enzyme is also of great importance in the bioactivation of mutagens, including the *N*-hydroxylation of arylamines. These enzymes are generally distinguished from CYPs in other families by their capacity to oxidize a variety of polynuclear aromatic hydrocarbons. The induction is mediated by a ligand-activated transcription factor, the aryl hydrocarbon receptor, (AHR), which binds to enhancer elements flanking the *CYP1A1*, *CYP1A2*, and *CYP1B1* genes and stimulates transcription (Shimada & Fujii-Kuriyama, 2004). This mechanism will be described below.

CYP1A1 and 1B1 are expressed in a wide range of extra-hepatic tissues and catalyze both the activation and detoxification reactions of PAH metabolism (Shimada & Fujii-Kuriyama, 2004). In addition, both enzymes are inducible by the PAH in cigarette smoke (Nebert et al., 2004). Induction of these two enzymes is generally mediated by the AHR, but differences may exist in the mode of induction for each enzyme.

Research on human hepatoma cells gene expression changes caused by BaP at 12 time points after exposure, in relation to DNA adduct and cell cycle, the temporal profiles for functional gene sets demonstrate both early and late effects in the up- and down-regulation of the relevant gene sets involved in cell cycle, apoptosis, DNA repair, and the metabolism of amino acids and lipids. Many significant transcription regulation networks appeared to be performed by transcription factors, TF, that are proto-oncogenes or tumour suppressor genes. Most correlations are with DNA adduct levels, which is an early response, and less with the later responses on G1 and S phase cells. The majority of the modulated genes are regulated by several of these TF, e.g., 73% by nuclear factor-kappa B and 34–42% by c-MYC, SRF, AP1, and E2F1. All these TF can also regulate one or more of the others. The data indicate that a complex network of a few TFs is responsible for the majority of the transcriptional changes induced by BaP. This network hardly changes over time, despite the fact that the transcriptional profiles clearly alter, suggesting that other regulatory mechanisms are also involved (Joost et al., 2010). These include the AHR pathway, for example.

3.2 The metabolism of tobacco smoke

Tobacco from both active and passive smoking has the main point of entry into the body via the airways; some constituents are dissolved in saliva and absorbed or swallowed. Virtually all organs and tissues are reached by the active products of smoking. Data from epidemiological studies confirm the widespread presence of active products of tobacco smoke on tissues and organs (Taioli, 2008). After binding the AHR, they induce Phase I and Phase II mRNAs and their corresponding proteins both *in vitro* and in experimental animals. The CYP superfamily members CYP1B1 and CYP1A1 have been reported to be expressed in human lungs. The highly reactive bioactivated intermediates of inhaled PAH carcinogens, such as benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide, confer "hot spots" for benzo(*a*)pyrene-7,8-dihydrodiol-9,10-epoxide-induced mutations in the tumour suppressor p53 gene *in vitro* and closely match the overall p53 gene mutation spectra found in a wide array of epithelial cancers *in vivo*. There is coordinate metabolism of estradiol and inhaled PAH by CYP1B1, suggesting a need to assay gender-associated factors impacting carcinogen metabolism expression in the human lung (Spivack et al., 2003).

The balance between the metabolic activation and detoxification of carcinogens varies among individuals and likely affects cancer susceptibility. Persons with a higher activation and lower detoxification capacity are at the highest risk for smoking-related cancers (Taioli, 2008).

4. The aryl hydrocarbon receptor in lung and the polycyclic aromatic hydrocarbons

The lung serves as a primary site for the xenobiotic metabolism of environmental toxicants and airborne pollutants. The lung is composed of more than 40 different cell types and is

known to activate pro-carcinogens (e.g. polycyclic aromatic hydrocarbons or N-nitrosamines) into more reactive intermediates that easily form DNA adducts (Pavek & Dvorak, 2008). Epidemiological studies suggest that exposure to PAH has been associated with an increased risk of lung cancer (Lin et al., 2003). The body has numerous molecular mechanisms that induce and restrict the activity of cell receptors in order to respond to these environmental compounds. These studies recognized the AhR (protein, AhR; gene, *AHR*) as a receptor that binds a wide variety of endogenous and exogenous compounds; it was identified as a mediator of the induction of certain xenobiotic drug metabolizing enzymes and the toxicity elicited by halogenated aromatic hydrocarbons (Gasiewicz & Collins, 2008). AHR is both a player in chemical toxicity and an important component of normal development (Nguyen & Bradfield, 2008). It was discovered due to its stimulation by a variety of planar aromatic hydrocarbons with BAP as prototype. Currently, more than 400 exogenous ligands have been identified. The best studied and one of the most potent ligands knows so far is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Dietrich & Kaina, 2010). Human lung bronchial-epithelial cells (NL-20) expresses mRNA of AHR (Fig 1A) with important levels of the protein delocalized in the cell (Fig 1B) and important translocation of it into the nucleus after exposition of solvent extractable organic matter (SEOM) 0.1 μg/μl (Fig. 1C). Interaction of the antagonist of the AhR induces both accumulation of the mRNA (Fig. 1D) and the protein (Figs 1E-1F).

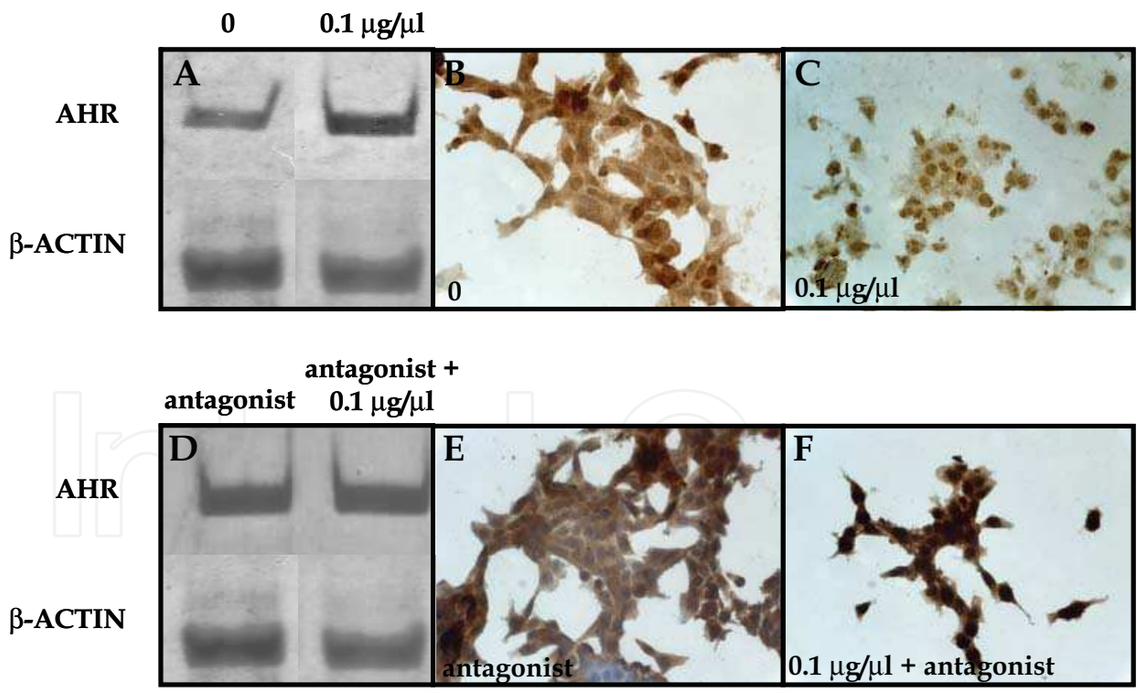


Fig. 1. Effect of PAH contained in SEOM on NL-20 human bronchial epithelial cells in the expression of the mRNA of *AHR* (A and D) and the protein AhR in cells exposed to 0.1 μg/μl of SEOM alone (from A to C), and in presence of SEOM plus an antagonist of the AhR (from D to F). PCR products resolved in polyacrylamide-gel electrophoresis and silver staining. Immunohistochemistry of AhR. Micrographies at 200X

AHR can be a molecular marker of exposition to different levels of air pollutants and it can reveal different populations exposed. Thus, residents in Mexico out of big cities as Mexico City (Fig. 2A) express high levels of the mRNA of the *AHR*. It trends to lowering if the lung is of Mexico City dwellers (Fig. 2B) and it decrease importantly in lung of active smokers (Fig. 2C). These results correlate with the cycle of the AhR, which is degraded if is interacting with its ligand (PAH) and it is maintained when there is no ligand present in the air. The main issue in these results is that mRNA of the *AHR* can be a molecular marker of air pollution, mainly to PAH in lung tissue aged for several years, 5 years as is illustrated in the Fig. 2.

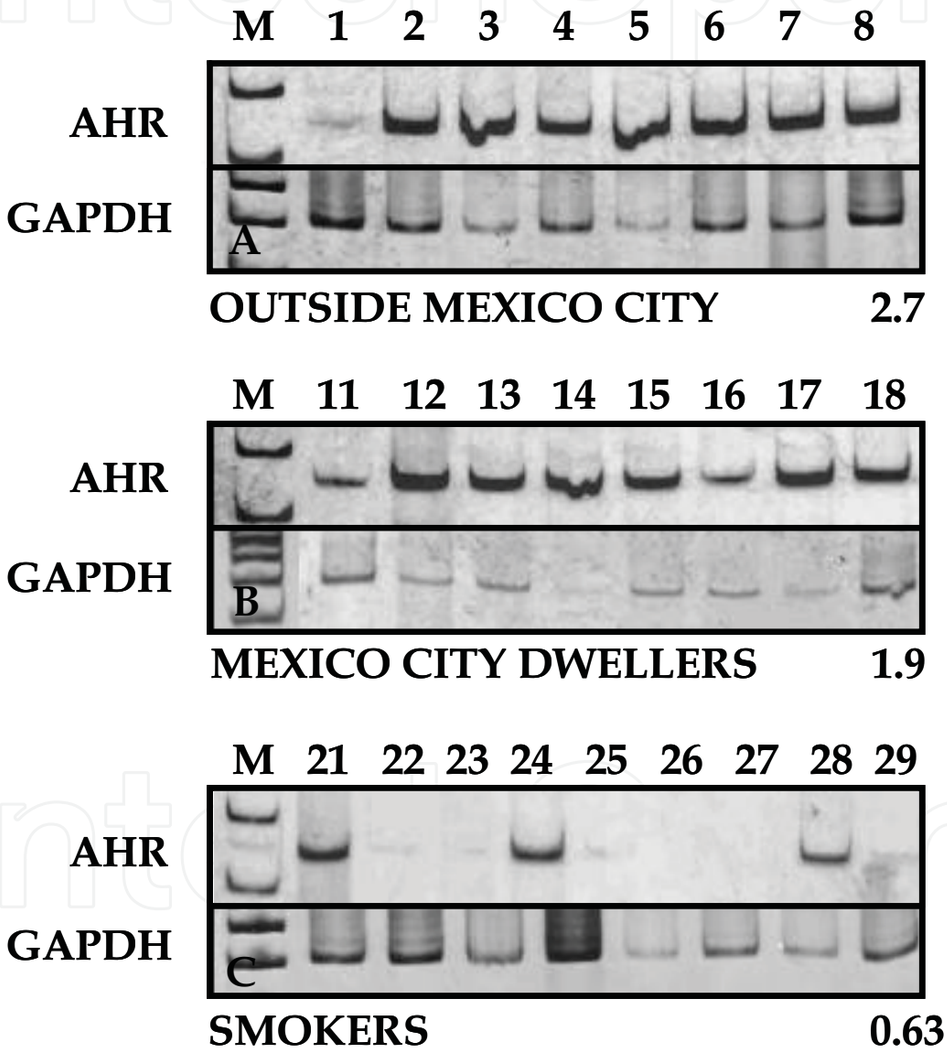


Fig. 2. RT-PCR of *AHR* in human lung tissue from outside residents (A), Mexico City residents (B) and in smokers (C). mRNA was obtained from paraffin embedded tissue. The number below of each PCR-gel indicates the relation of expression of the gene. GAPDH is gliceraldehyde phosphate dehydrogenase as gene control. Each lane is a different case. M, molecular markers. PCR products resolved in polyacrylamide-gel electrophoresis and silver staining

Finally, AHR is an important gene whose expression is observed along of the ages of the human been: from 40 hours post-natal (Fig. 3) to adults more than 60 years-old (some cases in Fig. 2).

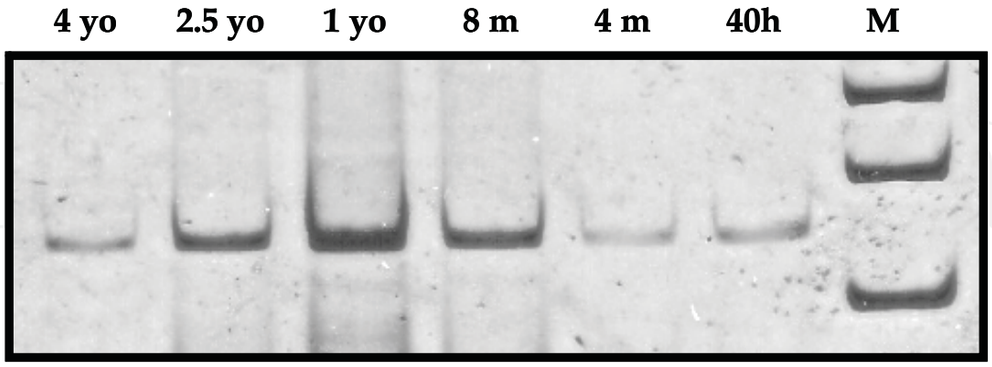


Fig. 3. RT-PCR of *AHR* in human lung tissue of children from 40 hours (h), 4 and 8 months (m) to 1, 2.5 and 4 years old (yo). M, molecular markers. RT of constitutive gene, glyceraldehyde-3-phosphate dehydrogenase was equal in each sample (not shown). PCR products resolved in polyacrylamide-gel electrophoresis and silver staining

4.1 The components of the AHR pathway

The AhR has been shown to bind, as have similar highly lipophilic halogenated and non-halogenated hydrocarbons, leading to cardiovascular, carcinogenic, and endocrine effects. At the cellular level, activated AhR interacts with various signal transduction pathways, induces biotransformation enzymes, alters the cell cycle, cell adhesion, and migration, and causes apoptosis or aberrant cell growth. *In vivo*, AhR plays significant roles in connection with development, immunological and reproductive functions, and adaptive responses to light and xenobiotics (Wincent et al., 2009). For these reasons, AhR regulates several important functions in the cell and is recognized by all participants as an AHR pathway.

AhR is a ligand-dependent transcription factor belonging to the basic helix-loop-helix/Per/ARNT/Sim (PAS) family, that regulates the expression of a battery of genes in a wide range of species and tissues (Dietrich, 2010; Gasiewicz & Collins, 2008).

The inducing chemical enters the responsive cell and binds with high affinity to the cytosolic AhR, which exists as a multiprotein complex containing two molecules of the chaperone protein hsp90 (a heat shock protein of 90 kDa) (Denison & Nagy, 2003), which is required to maintain the receptor in a conformation that facilitates ligand binding. The X-associated protein 2 [XAP2], also called AIP and ARA9, appears to function by stabilizing the interaction between hsp90 and the receptor, has some role in regulating the rate of AhR turnover, intracellular localization and interaction with other proteins in the nucleus (Gasiewicz & Collins, 2008), and an identified 23-kDa co-chaperone protein referred to as p23 (Denison, 2003) to stabilize an intermediary complex that contains the ligand-occupied hsp90-associated AhR (Gasiewicz & Collins, 2008). Binding of the ligand results in nuclear translocation of the AhR, dissociation from the chaperone proteins, heterodimerization with Arnt (Aryl hydrocarbon Receptor Nuclear Translocator) and the subsequent binding of the Ahr-Arnt heterodimer to dioxin-responsive elements (DREs) with the consensus core recognition sequence 5´- TNGCGTG-3´ (Fig. 4), also known as Xenobiotic-Responsive Elements (XREs)(Dietrich, 2010).

Arnt is a basic helix-loop-helix (bHLH) protein that also contains a PAS domain. In addition to forming heterodimers with many other bHLH-PAS proteins, including the AhR and hypoxia-inducible factors 1alpha, 2alpha and 3alpha, Arnt can also form homodimers when expressed from its cDNA *in vitro* or *in vivo* (Wang et al., 2006). AhR/Arnt binds with the TATA-binding protein (TBP) and several TBP-associated factors (TAFs), leading to general transcriptional machinery with RNA polymerase II (RNA pol II) and the transcriptional activation of target genes. The AhR is then exported to the cytosol and degraded by the 26S proteasome pathway. A mechanism of negative feedback regulation of the AhR function is performed by the aryl hydrocarbon receptor repressor (AhRR). Ligand-activated AhR/Arnt heterodimer transactivates the expression of target genes including the *AHRR* gene. AhRR suppresses AhR transcriptional activity by competing with AhR for dimerizing with Arnt and binding to the DRE sentence of target genes (Pavek, 2008) (Fig. 4). The interaction of the AhR with Arnt increases their capacity to bind specific enhancer sequences adjacent to target promoters called DREs. An assembly of coactivators and general transcription factors, including p300, SCR-1, p/CIP and transcription for IIB, then interacts with gene promoters and potentiates the expression of target loci (Nguyen, 2008).

4.2 The target genes of the AHR pathway

The interactions of PAH ligands with the AhR may explain the pathway of CYP induction (Lee, 2008) and several genes (Marley et al., 2005), thus leading to their detoxication and excretion and, at the same time, to their metabolic activation to genotoxic compounds (Dietrich & Kaina, 2010). The nuclear AhR complex, ligand-AhR-Arnt, interacts with consensus dioxin or XREs in the CYP 1 promoter and in promoters of other Ah-responsive genes, and the subsequent recruitment of coactivators and the general transcription of coactivator and general transcriptions factors results in the expression of target genes, such as genes phase I xenobiotic metabolizing enzymes (CYP1A1,1A2 and 1B1) and phase II enzymes (NQO1, GSTA2, UGT1A1 and UGT1A6) (Pavek & Dvorak, 2008) (Fig. 4).

Their action in the metabolism of PAH was explained in the previous section. Cyclo-oxigenase-2 (COX-2) has a XRE site in its promoter region, and PAH and TCDD induce COX-2 and prostaglandin synthesis (Marley, 2005). However, the mechanism by which AhR activation may result either in carcinogenic or protective effects is not clear. Moreover, many intracellular interactions of AhR and Arnt with various regulatory transcription factors, such as retinoblastoma protein-1, NF-kB, estrogen receptors and SP1, as well as with different coactivators and repressors, may modify the transcriptional activity of the AhR–Arnt heterodimeric complex, and this might explain why chemicals that bind to AhR may elicit detoxification agonist or antagonist responses (Irigaray P, 2010).

The roles of AhR in the carcinogenic process may be positive and negative. The ultimate response may be dependent on the level and length of exposure, as well as on the cellular context, stage of differentiation and the presence of other conditions in the tissue environment (Gasiewicz, 2008).

5. New molecular markers, microRNAs: definition and biogenesis

MicroRNAs or miRNAs are small non-coding RNAs almost 22 nucleotides long, involved in negative post-transcriptional gene regulation via the RNA interference mechanism. The sequences of miRNAs are highly conserved among plant-microorganisms-animals, suggesting that miRNAs represent a relatively old and important regulatory pathway. Up to

a third of the human genes are regulated by miRNAs. They are important regulators of several genes in many and broad biological processes, from developmental timing to cellular proliferation and apoptosis (Tomankova et al., 2010).

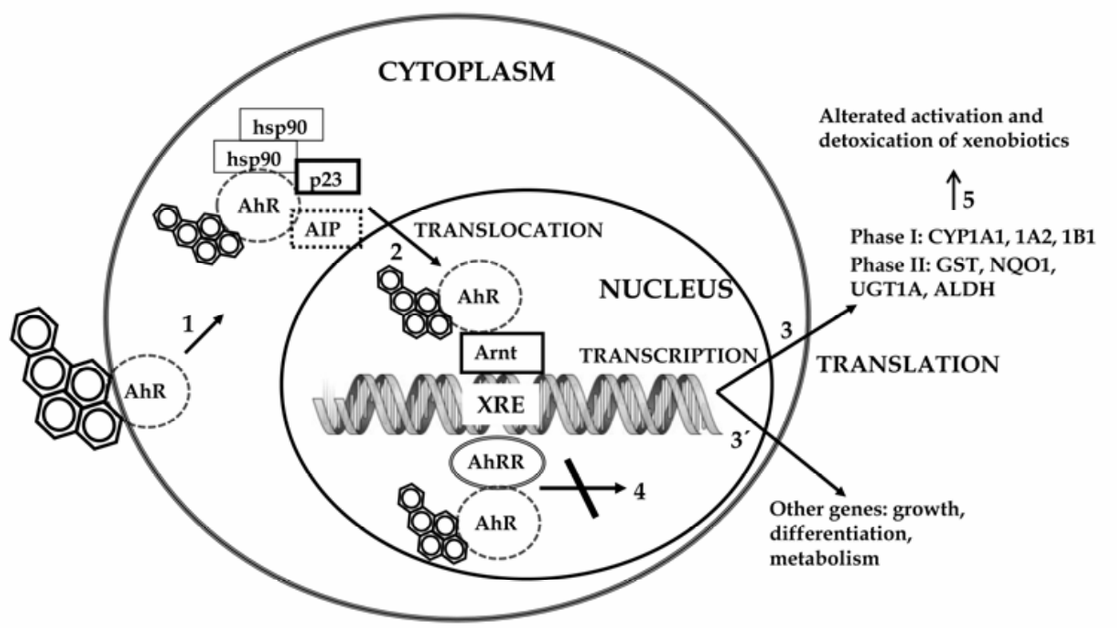


Fig. 4. Activation of the AhR and the AHR pathway. PAH interact with the receptor AhR (1) an it is assisted with other proteins as hsp90, p23 and AIP. AhR-PAH complex is translocated to the nucleus (2) and it makes complex with Arnt in order to activate transcription of several genes as Phase I and Phase II (3), and other genes participating in other cellular responses (3´). If AhRR interacts with the AhR-PAH complex, the transcription is inhibited (4). The transcription is performed in order to response the detoxification of xenobiotics and other molecules (5)

MiRNAs genes are localized in the non-coding regions, 3´, or in the introns or exons of protein-coding genes in the genomic DNA. They can be codified singly or in clusters. The miRNA genes are much longer than biologically active, mature miRNAs that originate through a multistep process (Fig. 5). They are transcribed by RNA polymerase II and lead to hundred- or thousand- nucleotides-long primary miRNAs transcripts (pri-miRNA) (Kim, 2005).
A local stem-loop structure of pri-miRNA is then cleaved in the nucleus by the dsRNA-specific ribonuclease Drosa/Pasha to the 70 nucleotide-long precursor miRNA (premiRNA). PremiRNA is actively transported from the nucleus to the cytoplasm. In the cytoplasm, pre-miRNA is subsequently cleaved by the RNase III Dicer into an almost 22-nt miRNA duplex. One strand of the miRNA duplex is degraded ("passenger, miR"), whereas the other is incorporated into the RNA-induced silencing complex (RISC) and serves as a functional, mature miRNA. Depending on the complementarity between miRNA and the 3´untranslated region (UTR), of the target mRNA there are two known mechanisms of miRNA action: 1) target mRNA degradation; and, 2) translational inhibition with little or no influence on mRNA levels (Fig. 5). The deadenylation and subsequent degradation of the target mRNA occurs when miRNA is near-perfectly complementary with target mRNA. This represents the major mechanism of miRNA regulation. About 84% of all protein-coding

mRNA targets undergo degradation when recognized by their cognate miRNA. Otherwise, the translational inhibition occurs when miRNA is only partially complementary to its target mRNA. This mechanism does not represent a predominant reason for reduced protein output. Several other factors may influence the action of miRNA, such as impaired processing, methylation, gene polymorphisms, gene amplification, deletion of the Dicer, translocations and others (Kim, 2005; Tomankova et al., 2010).

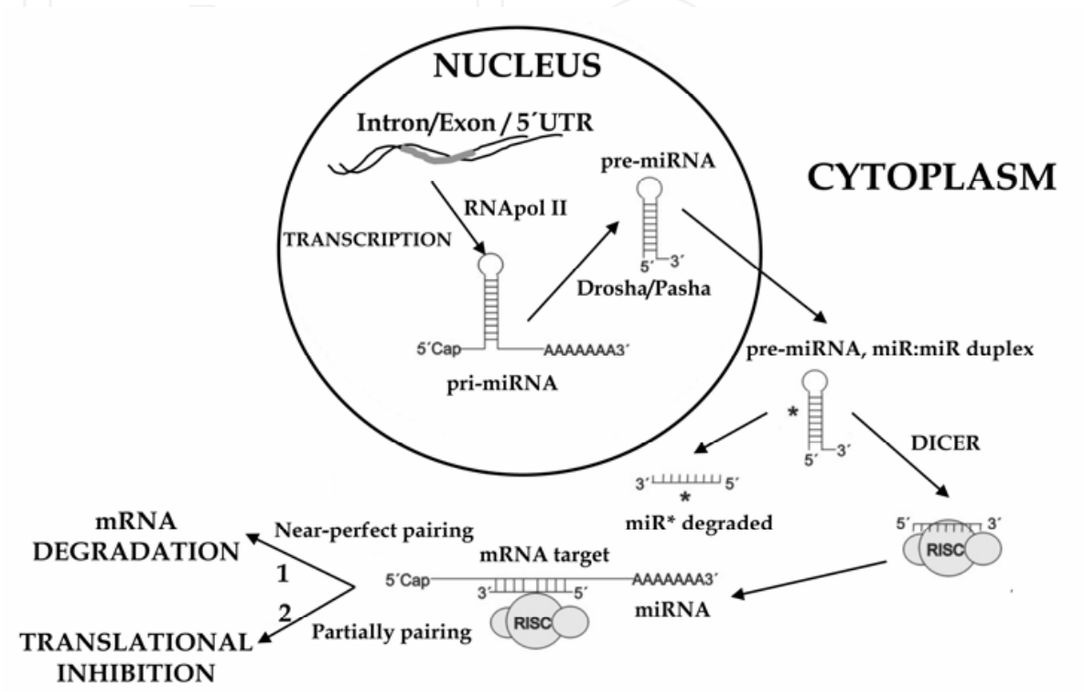


Fig. 5. MiRNAs are transcribed by RNA polII from genomic DNA as long primary miRNA transcripts (pri-miRNAs). Pre-miRNAs are then transported from the nucleus to the cytoplasm where is subsequently cleaved by RNase III Dicer into ~22 nt miRNA duplexes, consisting of the "guide" strand (miR) and"passanger" strand (miR*) which is degraded. MiR is incorporated into RISC and serves as a functional mature miRNA acting by two different mechanisms according to the complementarity with the target mRNA. According to Kim, 2005

It is evident that a single miRNA may regulate the translation of numerous downstream mRNA, and mRNA is likely to be regulated by several miRNAs simultaneously. The identification of miRNA target genes is important and due to high similarities in miRNA sequences, computational algorithms may predict a large number of putative miRNA binding sites on mRNA targets. Thus, experimental validation in biological systems is fundamental to completing the target prediction (Kuhn et al., 2008; Tomankova et al., 2010). Modulation of miRNA repression has been shown to be a highly dynamic process, as evidenced by studies that demonstrate the rapid alleviation of miRNA-mediated translational repression in response to specific needs.

5.1 The role of microRNAs in lung development

The lung has a very specific miRNA expression profile, which is highly conserved across mammalian species. The roles of miRNAs on physiological and pathological conditions in

the lung compartment are still limited and based mainly on studies of animal models. The increased expression of 8 miRNAs in neonatal and fetal lungs, miR-134, -154, -214, -296, -299, -323, -337 and -370; and the up-regulation of 5 miRNAs in the adult mouse and human lung, miR-26b, -29a, -29b,-142-3p and -187; were found to be conserved during development in both species. The examination of their genomic localization shows that 6 of the miRNAs are highly expressed in the developing lungs of mice and humans: miR-134, -154, -299, -323, -337 and -370, and map to the *Gtl2-Dio3* domain at human chromosome 14q32.21, a region that is highly conserved between the two species. Importantly, imprinting means that these miRNAs are only expressed from the maternally inherited chromosome and their expression is regulated by an intergenic germline-derived differentially methylated region located ~200 kp upstream from the miRNA cluster. Imprinting also results in the *Gtl-2* gene being expressed from the maternal chromosome (Williams et al., 2007).

MiRNA deregulation may contribute to various pulmonary diseases. Several miRNAs, such as miR-155, miR-26a, let-7, miR-29, miR-15/miR-16, miR-223, miR-146a/b and the cluster miR-17-92, have been shown to be involved in homeostasis and in lung development. For example, miR-26a has been shown to be selectively expressed in the bronchial and alveolar epithelial cells in the murine lung. MiR-29a and miR-29b are up-regulated in adult tissue and expressed at a lower level in developing lungs in mice and humans. These miRNAs genes map to human chromosome 7q32.3, a region that maps to a fragile site that has been associated with a number of cancers (Nana-Sinkam et al., 2009).

The miR-17-92 cluster is believed to regulate lung development because its expression is high in embryonic development and steadily declines during development into adulthood. Overexpression of the miR-17-92 cluster in murine models resulted in an abnormal phenotype manifested by the absence of terminal air sacs, which were replaced by highly proliferative, undifferentiated pulmonary epithelia. Finally, miR-223 has been shown to be crucial for normal granulocyte development and long function lung (Lu et al., 2007).

There is evidence that the up-regulation or down-regulation of miRNAs is critical for lung development/homeostasis and thus may contribute to the development of pathological pulmonary conditions, smoking-related diseases including lung carcinogenesis, fibrosis and other disorders, such as allergies. The effect of such environmental factors as organic pollutants and PAHs emitted from burning food or produced by the incomplete combustion of fossil fuels in automotive engines can be studied. Some can be highly specific, such as the reduced miR-146a expression that results in a prolonged mRNA half-life of cyclo-oxygenase-2 that increases prostaglandin E2 in the fibroblast of COPD (chronic obstructive pulmonary disease) subjects. However, the majority of miRNA studies in smoking-related diseases focus on the role of miRNAs in lung cancer (Tomankova et al., 2010).

5.2 Responses of microRNAs to smoking

The three PM can induce a response by the lung in inhabitants of large cities that can activate several molecular responses. The first lung response evaluated has been that due to smoking. Smoking induces the expression of airway genes involved in the regulation of oxidant stress, xenobiotic metabolism and oncogenesis, while suppressing those involved in the regulation of inflammation and tumour suppression. This pattern can be described in cytologically normal airway epithelial cells, thus these airway genes can serve as biomarkers for lung cancer and even in the early stages of neoplasic transformation and, of course, in other diseases (Spira et al., 2007). These studies suggest that gene expression changes in the airway epithelium reflect host responses to, and damage from, cigarette smoke.

In the case of microRNAs responses to smoking, Schembri et al. (2009) examined whole-genome microRNA and mRNA expressions in bronchial airway epithelia of current and never smokers, finding 28 miRNAs to be differentially expressed with the majority being down-regulated in smokers. Previously, 26% of the mRNAs that are differentially expressed in smokers were predicted to be targets of only these 28 miRNAs/miRNAs families. This suggests that a relatively small number of miRNAs in response to smoking could potentially contribute to several of the smoking-associated changes in mRNA expression, and that modulation of specific miRNAs might therefore represent a mechanism that contributes to the overall host response to tobacco smoke exposure. Gene ontology analysis of the anti-correlated targets of these miRNAs reveals that these genes are involved in cell-to-cell adhesion, cellular signalling and the cytoskeletal structure. These findings must be confirmed by other studies. Using two strategies, one from the mRNA microarrays expression dataset of normal human bronchial epithelial cells exposed to cigarette smoke, and the other by the exposure to cigarette smoke condensates of normal human bronchial epithelial cells, both studies indicated that the targets of mir-128 are induced upon acute exposure to cigarette smoke and suggest that mir-128 expression likely decreases upon such exposure (Fig. 6). Manipulating the levels of mir-128 by several strategies that increase or decrease its levels indicates that they modulate the airway epithelial gene expression response to cigarette smoke and supports a role for miRNAs in regulating host responses to environmental pollutants. The finding that most miRNAs were differentially expressed, with most (82%) being down-regulated in smokers, is similar to studies of miRNA in cancer. In both cases, most miRNAs were down-regulated when compared to normal tissues or cells from never smokers. This may be because miRNA levels are closely linked to the degree of cellular differentiation and because the reduced expression of miRNAs in cancer is associated with more dedifferentiated tumours, and the same alteration in the bronchial cells of smokers. These results suggest that the down-regulation of miRNAs in smokers could be associated with the development of tobacco-related cancers. Airway miRNA expression could potentially serve as an indicator of smoking-induced disease processes. Using experimental methods, Schembri *et al.* (2009) performed an experimental validation in biological systems of the role of mir-128 in regulating the expression of transcriptional regulators such as MAFG. These findings suggest that miRNAs may play a role in regulating the gene expression response to tobacco exposure in airway epithelial cells. Finally, the analysis of miRNAs in lung cancer in never smokers, is important to show the molecular characteristics of lung cancer in never-smokers: changes in the expression of a relatively small number of miRNAs are involved in lung carcinogenesis, miR-138 is down-regulated preferentially and miR-21 is one of the most aberrantly increased miRNAs in both never smokers and cases of smokers.

5.3 Responses of microRNAs against 3, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK)

Lung cancer is the most common cause of cancer deaths in the world. Approximately 90% of all lung cancers are directly attributable to smoking. Animal models are invaluable tools for studying the initiation and progression of human disease. To mimic tobacco carcinogenesis, Kalscheuer et al. (2008) used a male model with F344 rats that were chronically treated with 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), a carcinogen present in tobacco products, for up to 20 weeks. NNK along with polycyclic aromatic hydrocarbons are the most prevalent and potent carcinogens in tobacco products and smoke. Microarray analyses of miRNA expression revealed that a small number of miRNAs, such as miR-101, miR-126*,

miR-199a, miR-199b and miR-34b, were down-regulated in NNK-treated rats when compared with controls. These results were also confirmed by Northern blot and real-time PCR, at least for miR-34b, miR-101, miR-126 and miR-199a.

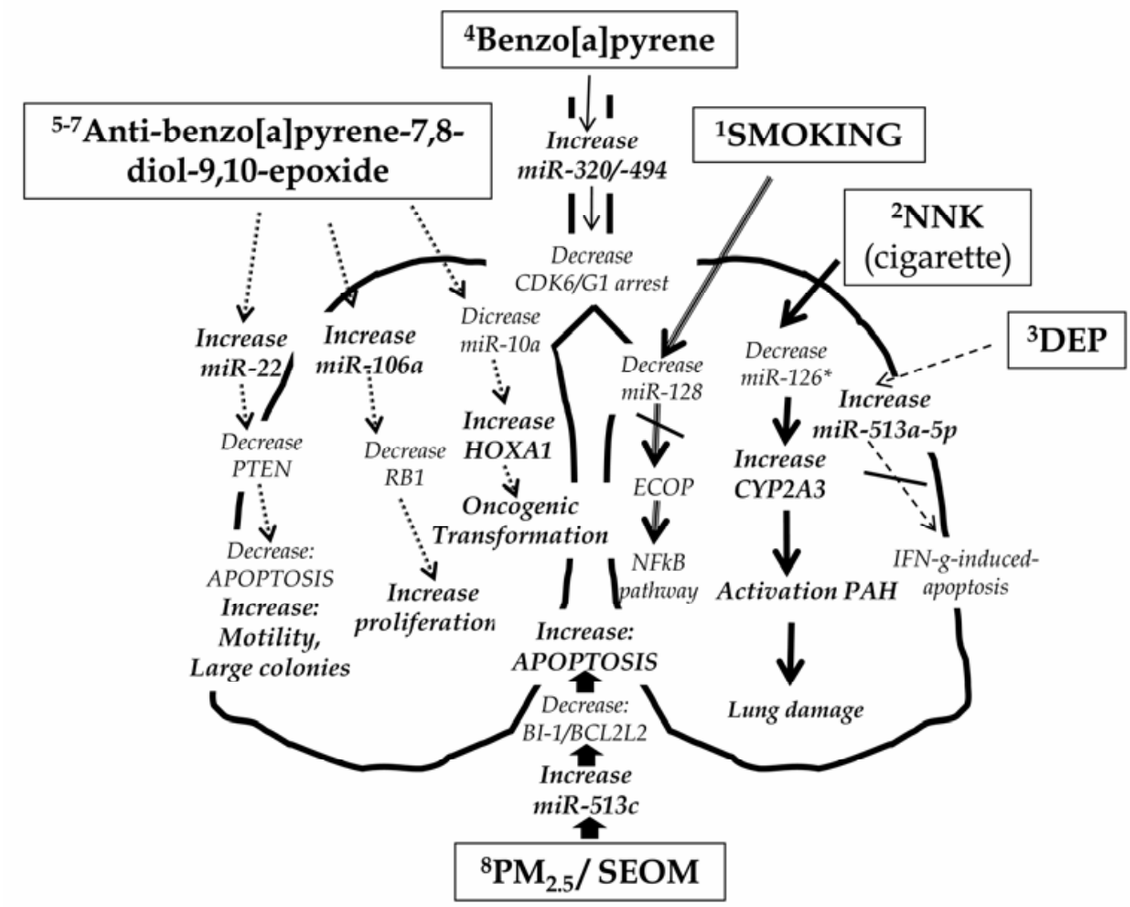


Fig. 6. Involvement of miRNAs in lung responses to several PAH from the DEP, SEOM of $PM_{2.5}$ or other carcinogen PAH of the tobacco smoke. When the microRNAs are induced (bold), the response is to degrade mRNA of some target genes. This cellular response is inhibited/not induced. Otherwise, if the effects of pollutant induce a degradation of the microRNAs, the cellular response is activated/sustained (bold). This figure integrates the information of [1]Schembri et al., (2009); [2]Kalscheuer et al., (2008); [3]Jardim et al., (2009); [4]Duan et al., (2010); [5]Jiang et al., (2011); [6]Liu et al., (2010); [7]Shen et al., (2009) and [8]Amador-Muñoz et al., (2011) and Miguel-Perez et al., (2011) results not published

In order to validate one of these findings, human and other species of *CYP2A13* genes were shown to be prominently predicted as targets by for miR-126*. Deleting much of the predicted miR-126*-binding site in the 3´UTR abrogated the inhibitory effect of miR-126 on reporter expression, demonstrating the specificity of the interaction between the C*YP2A3* mRNA of the rat and miR-126*. This model mimicked the early stages of lung cancer development. These miRNAs could indicate early biomarkers for lung cancer. Many miRNAs might not change their expression levels until later stages of pulmonary

tumorigenesis. The finding that miR-126* may regulate the expression of C*YP2A3* suggests that the early changes in miRNA expression indeed have important biological consequences (Fig. 6). Finally, miR-34 is another miRNA with clearly interesting implications due to the fact that it inhibits the expression of a large number of genes involved in DNA damage response, cell cycle progression and apoptosis.
The results of this study open the possibility that some of these miRNAs and the target genes or pathways regulated by the miRNAs might constitute early diagnostic markers for human lung cancers.

5.4 Responses of microRNAs to Diesel Exhaust Particles (DEP)

Jarim et al. (2009) revealed a disruption of the expression of microRNAs in human airway cells exposed to 10 mg/cm² of DEP for 24 h. They analysed total RNA for miRNA expressions using microarray profile analysis and quantitative real-time polymerase chain reactions. DEP treatment causes differential expression patterns of miRNAs in differentiated human bronchial epithelial cells. A total of 130 miRNAs showed an increase of ≥-1.5-fold in response to DEP, whereas in the case of smoking, 67 showed a decrease ≥ 1.5-fold in expression.
After an analysis of the miRNAs that were more altered in their expression, those authors found that a family of miRs, such as miR-513c, miR-513b and miR-513a-5p, are over-expressed 16-, 13- and 11.3-fold than controls. Other miRNAs, miR-923, miR-494 and miR-338-5p, had 10.6-, 9.2- and 4.6-fold expressions. On the other hand, the lowered miRNAs were miR-31, miR-26b, miR-96, miR-27a, miR-135b and miR-374a. Several functions related to lung physiology/response are involved, such as miR-513a-5p, in the repression of IFN–γ–induced apoptosis, and miR-26b was down-regulated in the lungs of rats exposed to cigarette smoke (Fig. 6). MiR-96 has been shown to regulate levels of the protein arginine methyltransferase (PRMT5), which interacts with the chromatin remodelling complex and may play a crucial role in silencing tumour suppressors. Identifying the network analysis of miRNAs highly modulated by DEP, using TargetScan and miRDB for highly modulated miRNAs, reveals molecular networks that were enriched by inflammatory responses that correlated well with canonical signalling pathways, such as interleukin (IL-8), nuclear factor kappa B (NF-kB), and the chemokine (C-X-C motif) receptor 4, where these targets could be acting. To further analyse the putative effects of differential miRNA expression profiles due to DEP exposure, Jarim *et al.* used a TargetScan and miRDB analyses to identify possible mRNA targets.
There are different types of DEP, all of which have been found to possess mutagenic potential. Although DEP are classified as potential causal carcinogenic agents, the mechanism by which they alter cellular function remains largely unknown. Furthermore, the carbonaceous core of DEP alone can induce tumour formation (Wichmann, 2007). Future studies will show whether aberrant DEP-induced miRNA expression changes can be functionally linked to tumorigenesis

5.5 Responses of microRNAs to Benzo[a]pyrene, BaP

There is an important work group at the Institute for Chemical Carcinogenesis of the Guangzhou Medical University in China that has described several miRNAs in *in vitro* models of exposure to PAH. When Duan et al (2010) exposed with 1 mM BaP for 24h, they revealed an important increase in miR-320 and miR-494 expression. Since carcinogenesis is considered to be the result of the deregulation of the cell cycle machinery, the transitions between cell-cycle phases are mediated by cyclin-dependent kinases and their modulators; and little is known about the functions of miRNAs in the process of BaP-exposed cell cycle

alteration. In this sense, when primary murine bronchial epithelial cells were exposed to BaP, the analysis of the cell cycle progression revealed that G1 arrest occurred in response to BaP. This depends, at least partially, on reduced expression levels of CDK6. These results were confirmed using antago-miRs corresponding to miR-320 and miR-494. Some important additional data are that miR-494 is highly expressed in retinoblastoma and several solid tumours. Central to these events is the G1 arrest, which allows cells more time to repair DNA and/or bypass the machinery time to remove or resolve the damaged sites before the DNA synthetic phase of the mammalian cell cycle, and protects cells from mutagenesis. In this sense, G1 arrest may be related to the cellular defence mechanism, which can arrest the progress of the cells with damaged DNA through the cell cycle, prior to their entry into a critical phase, such as DNA replication. In conclusion, CDK6 may be a target of miR-320 and miR-494 affecting G1/S transition through this cycle (Fig. 6).

5.6 Responses of microRNAs to anti-BPDE and miR-106a

The polycyclic aromatic hydrocarbon BaP, which is a toxic element in the environment in general and in tobacco smoke in particular, has atherogenic and carcinogenic properties. BaP is activated by microsomal enzymes to yield anti-benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxide (anti-BPDE), which binds covalently to nuclear DNA to form adducts that can initiate carcinogenesis. A malignant transformation model of the human bronchial epithelium cell line 16HBE induced by anti-BPDE was established by Jiang *et al.* (2011). The immortalized 16HBE cell line retains the specific morphology and function of normal human bronchial epithelial cells and provides a suitable resource for studying the molecular pathogenesis of lung cancer. Previous studies from this laboratory revealed 55 significantly differentially expressed miRNAs, identified by microarray in transformed 16HBE cells. This description suggests that some of these miRNAs may play important roles in the process of transformation due to anti-BPDE.

The levels of miR-106a were 2.9-fold higher in 16HBE transformed cells than in non-transformed ones. This effect produces a decrease in the proportion of cells in G1/S (40.11%) that is greater than that observed in normal cells (70.62%). Also, the apoptosis rate was different between the two kinds of cells, as the transformed cells had 4% of apoptosis, in contrast to more than 8% observed in non-transformed cells. The next step, the capacity for colony formation, was evaluated and the results showed important changes in the number of colonies from 8.39 for transformed cells to 5.79 for non-transformed ones. All these results support the suggestion that miR-106a affects cell proliferation, cell apoptosis, and colony formation in 16HBT transformed cells. Finally, the formation of xenografts was induced subcutaneously in BALB/c nude mice and they were measured after 7 days post-inoculation. The tumours derived from anti-miR-106a-transfected cells grew substantially more slowly compared to the transformed group. The tumours were harvested 42 days post-injection and the weight of the tumours from the anti-miR-106a-transfected cells was significantly less than that of those derived from transformed cells (Fig. 6). The assays validated the role of miR-106a in the tumorigenesis induced in the *in vivo* model.

Bioinformatic analysis showed that the tumour suppressor RB1 is one of the predictive targets of miR-106a. The levels of RB1 mRNA and protein in the transformed cells were below those of the cells transformed by anti-miR-106a, and vice versa. In a dual-luciferase assay, the relative luciferase activity of the reporter that contained the RB1 3´-UTR was markedly decreased compared to that seen with the parent containing the mutant sequence. These results showed that the effects of miR-106a were mediated by sequences in the 3´-UTR

of RB1 mRNA, which is in concordance with the results of previous studies. Over-expression of miR-106a provides a proliferative advantage to the malignant transformation of cells. The retinoblastoma tumour suppressor gene product RB1 regulates differentiation, apoptosis, and cell cycle control by coordinating the cell cycle at G1/S with transcriptional machinery. Therefore, miR-106a might function as an oncogene and might serve as a potential target for cancer therapy.

5.7 Responses of microRNAs to anti-BPDE and miR-22

Smoke contains many carcinogens and of course PAH is one of them and is wide-spread environmental pollutant found also in DEP and charbroiled food. Anti-BPDE is one of the many metabolites of BaP and is capable of electrophilic attacks on guanine residues, forming DNA adducts identical to those produced by BaP. Based on previous information, Liu et al. (2010) used bioinformatic tools to predict miR-22 target sites on PTEN (Phospatase and ensin homologue deleted on chromosome 10), and experimental analysis was used to validate the importance of these molecules in malignancy. MiR-22 was chosen because it had the highest predictive scores. The PTEN mRNA contains one binding site that is partially complementary to miR-22 and carries sequences identical to those of humans, mice, rats and other mammals. QRT-PCR analysis showed that miR-22 was significantly up-regulated as compared to normal cells. A similar pattern was observed by miRNA microarrays. The PTEN protein level in 16HBE-transformed cells was reduced significantly, but the PTEN mRNA level between normal and transformed cells was unchanged. Using anti-miR-22 over-expression in transformed cells revealed an increase of PTEN protein in those cells and the opposite effect was observed as the over-expression of miR-22 decreased the expression of the PTEN protein by almost half. Dual-luciferase assays proved that PTEN is a target of miR-22. These results indicated that miR-22 modulates the post-transcription expression of PTEN. MiR-22 also regulates other cellular processes, such as apoptosis, cell colony formation and motility. Increased caspase-3/7 activity was found for anti-miR-22 in 16HBE cells transformed by anti-BPDE. Under fluorescence staining, the inhibition of miR-22 in cells transformed increased the number of apoptotic cells in comparison to control cells. Cells transformed by anti-BPDE, with transfection with anti-miR-22, induced both markedly smaller colonies and a lower motility in the wound-scratch healing assay. In summary, miR-22 is increased in 16HBE cells transformed by anti-BPDE; this fact resulted in a decreased expression of PTEN, leading to resistance to apoptosis but increasing colony formation and cell motility. Cell motility reflects a cell's invasive capacity (Fig. 6).

5.8 Responses of microRNAs to anti-BPDE and miR-10a

In the same line of research, anti-BPDE is the most important metabolite of BaP activation. The study of the molecular mechanism of cell transformation induced by anti-BPDE was performed in 16HBE transformed-cells. In their study, Shen et al. (2009) quantified by QRT-PCR and found that the expression of miR-10a in 16HBE-transformed cells was 0.01 times that observed in non-transformed cells: it decreased. Previously, the RNA target of miR-10a was predicted by bioinformatic analysis and located within the HOXB cluster on 17q21, and was associated with the risk of human megakaryocytopoiesis and adult acute myeloid leukaemia. It also revealed HOXA1 as a target for miR-10a. QRT-PCR results demonstrated that HOXA1 mRNA expressions were 8.75-fold greater than those of normal 16HBE cells. Those findings do not validate the role of miR-10a in high expressions of the HOXA1 gene,

but it has been found that the forced expression of HOXA1 dramatically increases the anchorage-independent proliferation of immortalized human mammary epithelial cells. The down-regulated miR-10a and its up-regulated predicted target HOXA1 were expressed reciprocally in 16HBE-transformed cells, suggesting that miR-10a is potentially involved in cellular transformation, and might act as a candidate tumour suppressor by intervening in HOXA1 (Fig. 6).

5.9 Responses of microRNAs to soluble extractable organic matter (SEOM) of the $PM_{2.5}$

The organic chemicals associated with airborne particles can be evaluated through extraction, which generates solvent extractable organic matter (SEOM) that contains hundreds of compounds, among which the PAHs have been the most widely investigated in studies exploring the mutagenic and potentially carcinogenic activity of ambient particulate matter, mainly in Mexico by the Villalobos-Pietrini group (Villalobos-Pietrini et al., 2006). $PM_{2.5}$ contains PAH and Mexico City has important emissions of these particles due to mobile sources represented by four million vehicles and 35,000 industries in the Metropolitan Zone of the Valley of Mexico (MZVM). The concentrations of BaP and other PAHs and nitro-PAH are different depending of the zone of the MZVM, as the northeast, central and southeast zones have higher concentrations than the others: 0.404, 0.455 and 0.452 ng/m^3, respectively. However, benzo[*ghi*]perylene is the most abundant PAH in Mexico City's atmosphere with high levels of 1.84 ng/m^3 in the central zone and similar concentrations in the others. The C_{24}-C_{26} were the most abundant n-alkanes and 2-nitrofluoranthene and 9-nitroanthracene the most abundant nitro-PAHs (Amador-Muñoz et al., 2011). Our group evaluated the genetic responses of miRNAs due to exposure *in vitro* of human bronchial cells, NL-20, to SEOM in concentrations of 13 and 17 µg/ml for 24h. These concentrations of SEOM were proved to be mutagenic in a *Salmonella typhimurium* assay previously obtained of PM10. From several microRNAs evaluated, we demonstrated that there was an increase in the expression of miR-513c (Figure 7), due to SEOM obtained from a filter sample from the northeast station of the MZVM (San Agustín). This was similar to the results obtained by Jardim et al. (2009), which reported a 16-fold increase of miR-513c in primary human bronchial epithelial cells cultured and exposed to 10 mg/cm^2 of diesel exhaust particles (DEP). MiR-26b did not change after exposure. The evaluations of several miRNAs related to PAH response of the lung is currently under study in our laboratory using this *in vitro* model. An important response of NL-20 cells is that they suffered apoptosis, as revealed by the DNA-ladder after exposure for 24 h. After analysis, possible mRNA targets of miR-513c revealed by Targetscan are BCL2L2 (BCL2-like2) and BI-1 (Bax inhibitor-1) that have anti-apoptotic functions. One possible mechanism is miR-513c, which induces a degradation of one of these proteins (or both) and turns on apoptosis induction verified after SEOM exposure in NL-20 cells (Fig. 6). Currently, we are evaluating the responses *in vitro* in human bronchial cells after exposure to SEOM obtained from several monitoring stations, in order to view the differences in the molecular responses: miRNAs and AHR pathway activation, since $PM_{2.5}$ in the northwest and the southeast originates mainly from primary emissions of primary organic compounds. $PM_{2.5}$ in the northeast, central and southwest contains a greater proportion of secondary organic compounds, with the less oxidized organic aerosols being found in the northeast and the most aged organic aerosol in the southwest.

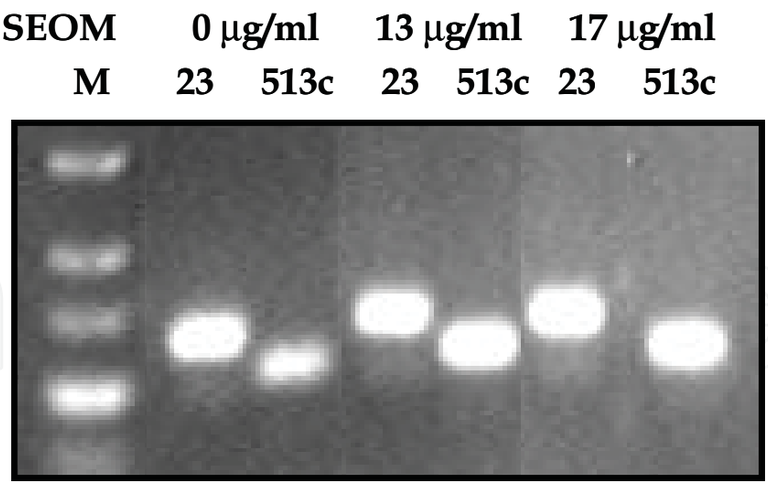


Fig. 7. Expression of miR-23 and miR-513c in human bronchial epithelial cells NL-20 exposed to 13 and 17 μg/ml of SEOM extracted of $PM_{2.5}$ from San Agustín, Northeast of México City. An important induction of miR-513c is evident. PCR products resolved in agarose 4% stained with ethidium bromide

6. Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution

6.1 Background and levels of exposure in several scenarios

The main sources of human exposure to PAH are occupational, passive and active smoking, food and water, and air pollution. The total intake of carcinogenic PAH in the general population has been estimated at 3μg/day. In smokers, BaP levels range from 0.5 to 7.8 μg/100 cigarettes when exposure is from mainstream smoke. Levels from passive smoking are lower, from 0.0028 to 0.76 μg/m^3. There is a high variation in atmospheric PAH levels across geographical areas, with BaP concentrations from 0.01 to 100 ng/m^3. In Mexico City, the mean BAP determined in 2006 was 0.3912 μg/m^3. Pollution of air by PAH is mainly due to the incomplete combustion of wood or fuel used for industrial or motor vehicle exhaust. The level of exposure to PAH through these sources is low compared to other sources, such as diet, occupation, or tobacco smoke. The half-life of airborne PAH is on the order of days, but can be longer when they are bound to small particles. Inhaled PAH are absorbed mainly thorough the bronchial epithelium. After absorption, PAH are distributed to tissues where they are biotransformed by the enzymes of phase I to chemically reactive intermediates that may bind covalently to DNA-producing DNA adducts. These give rise to mutation and, eventually, tumour initiation. PAH metabolites are mostly conjugated with glucuronic acid by phase II enzymes and excreted as hydroxylated metabolites. PAH are excreted mainly through the faeces; only about 10% are excreted in the urine (Castaño-Vinyals et al., 2004).

The difficulty in finding an index substance arises from the fact that the composition of PAH mixtures depends on the source of combustion. The most common compound used as a reference substance for carcinogenic PAH is BaP. Certain PAH and nitro-PAH are more important and characteristic of the environment of each city. For example, benzo[*ghi*]perylene was the most abundant PAH contained in the SEMO extracted from the $PM_{2.5}$ in Mexico City (Amador-Muñoz et al., 2011).

6.2 Biomarkers of PAH exposure

The most commonly used biomarkers of PAH exposure are metabolites of PAHs, particularly 1-hydroxypyrene (1-OHP) and DNA-adducts. 1-OHP is the principal product of pyrene metabolism, representing 90% of its metabolites (Brzeznicki et al., 1997). Following inhalation, the half-life of 1-OHP is on average about 18-20 hours (Buckley & Lioy, 1992). PAH adducts have been mainly employed as a measure of PAH linked to DNA in target tissues and cells. The half-life of DNA adducts in lymphocytes is on the order of months. There are protein adducts of PAH, such as albumin adducts whose half-life is around 20 days, while for haemoglobin adducts it is around 120 days (Castaño-Vinyals et al., 2004).

Occupational studies and research on active and passive smoking have reported positive correlations between BaP and some markers, such as 1-OHP and DNA-adducts, but in exposure situations where BaP concentrations were 10-to-100-fold higher than those normally found in ambient air concentrations (Dor et al., 1999). Castaño-Vinyals et al. (2004) demonstrated in their work, which integrated the highest number of studies quantifying biomarkers of PAH exposure from air pollution, that both 1-OHP and DNA-adducts can be usefully applied to assess environmental exposure to PAH. The correlation between environmental levels and 1-OHP was high; in particular when personal monitoring of BaP was done, indicating that this biomarker can distinguish between fairly small exposure gradients. For DNA-adducts, the studies analysed showed heterogeneity in several variables that probably resulted in an underestimation of the correlation between atmospheric PAH and DNA adducts.

6.3 Main markers of biomarkers of PAH exposure

PAH in the air are present at concentrations from tens (or less) of ng/m^3 in non-polluted or low-polluted areas to hundreds of ng/m^3 in polluted areas. Most studies have used BaP as a reference substance for their carcinogenic potential. However, the presence of other PAH in the environment, some of which are carcinogenic, does not allow an accurate estimation of the risk linked to a PAH mixture on the basis of BaP concentrations alone (Castaño-Vinyals et al., 2004). The major contributors to air PAH in the urban and suburban atmosphere are mobile sources, such as diesel and gasoline engines. Emissions from these sources contain mainly benzo[g,h,i]perylene, fluoranthene, and phenanthrene, so that measuring only BaP as an index substance may result in underestimating exposures. Such is the case of Mexico City, whose atmosphere contains concentrations of benzo[*ghi*]perylene (Amador-Muñoz et al., 2011) as high as those of other large cities like Rome (Cecinato et al, 2008) and Nanjing, China (Wang et al., 2006).

The correlation among different studies indicates that differences as low as 5 ng/m^3 of BaP measured through personal monitoring can be identified using these biomarkers. In this kind of study it is important to register several data, such as smoking status, dietary intake mainly in non-smokers and differences in the season of blood-drawing, all of which may be factors that affect results. The time of year is important because, in general, BaP levels in winter are higher than those measured during summer (Castaño-Vinyals et al., 2004).

The use of biomarkers to assess exposure to PAH at high levels is well-studied, but more work is needed to validate these biomarkers when exposure occurs at low environmental levels. At the group level, DNA adducts and, particularly, 1-OHP seems to reflect exposure well, even at low levels of air pollution. In contrast, protein adducts do not yet appear to be valid markers for assessing environmental exposure to BaP. The use of these biomarkers should be more widely implemented in combination with more traditional techniques to evaluate the effects of ambient air pollution (Castaño-Vinyals et al., 2004).

6.4 MicroRNAs as "new" or "complementary" biomarkers of PAH exposure

Due to the rapid and sustained response of the pulmonary cells to several different stimuli, such as PM exposure and also to PAH contained, molecular expression can be considered also as a way to response to PAH exposure and other contaminants of the air, and due to sometimes they are sustained in their expression for the PAH exposure, they could have a sensitivity as DNA-adducts has. MicroRNAs are molecules with a rapid and sustained response. They can be considered as biomarkers because:

1. they are induced in a specific way, according to the type of agent or contaminant
2. they can reveal cellular responses altered due to exposure
3. they can define a group of genes altered by the action of the microRNAs
4. they can belong to a specific group of microRNAs codified in fragile sites related to neoplasic diseases
5. the altered gene and cellular pathways allow us to predict the possible implications of several diseases, including cancer.

MicroRNAs are specific and, due to this property, we can confirm whether tissue or cells were exposed to some kind of contaminants in the air. For example, according to the studies performed by several authors, we can define one of them as a marker; e.g. miR-101, that was reported as a microRNA specific to exposure to isolated carcinogenic compounds of cigarettes (Kalscheuer et al., 2008). This miR normally decreases its expression in the presence of the tobacco carcinogen and increases its expression in cells not so exposed. We demonstrated that this miR-101 is expressed in lung tissue from non-smokers who lived in the 70s in Mexico, and its expression is increased (Fig. 8), thus confirming their lack of exposure to tobacco. We are using this molecular marker in order to confirm whether people were not really exposed. In that time, the passive smokers were frequent before the establishment of laws more strict of the tobacco in Mexico, and some cases can reveal low levels of miR-101 (Fig. 8, lanes 1 to 2).

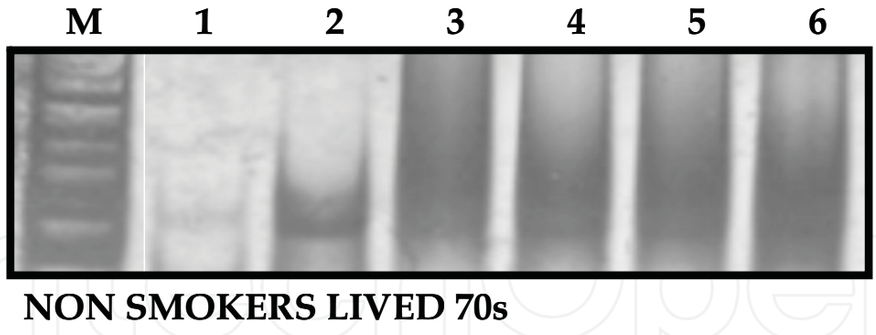


Fig. 8. Expression of miR-101 in lung tissue from people non-smoker lived at 70s in Mexico (From 1975 to 1978). Total RNA was extracted and the RT-PCR was performed to mir-101. Lines 3 to 6 have a lot of miR-101 amplification which explain the no definition of the band. Cases 1 to 2 showed less amplification of miR-101, maybe they were passive smokers. Each lane is a different case. M, molecular markers. PCR products resolved in polyacrylamide-gel electrophoresis and silver staining

7. Future research

The search for DNA adducts is problematic in terms of the quality of the DNA and its preservation, the kind of fixative, long time periods of preservation and the integrity of the

DNA in the cells. The use of paraffin-embedded tissue represents an easy, economical and practical form of preserving biological samples, and performing studies of the impact of climatic change and several other analyses. PriRNAs are RNA duplex that give stability. They can be stored in P-bodies and favour their preservation. They can be present in extracellular vesicles and maybe intracellular ones. These properties preserve their structure and permit their isolation in optimal conditions. They do not demand sophisticated, extra requirements or procedures, other than those that are standard in analyzing expressions following the general methodology of Molecular Biology. To consolidate the measurement of the levels of microRNAs, quantitive RT-PCR improve the results and analysis of its study. Some of the microRNAs described in Fig. 6 can be used as probable new molecular markers associated to PAH exposure in urban air. Also the information described by Schembri et al., (2009); Kalscheuer et al., (2008); Jardim et al., (2009); Duan et al., (2010); Jiang et al., (2011); Liu et al., (2010) and Shen et al., (2009) is important to be considered in future analysis of this kind of markers.

We can not leave the expression of *AHR* gene and AhR due that both have demonstrated be a sensitive and specific molecular sensors of the PAH exposure in urban air. Future work has to be started in other genes of the AHR pathway and it could explain the response of the target genes widely studied as CYPs genes due to PAH contained in urban air.

Finally, the information obtained from studies analyzing microarrays of microRNAs in response to particular air contaminants is important, because we can elaborate a guide of microRNAs with specific responses in the pulmonary cells against PAH, tobacco metabolites, tobacco carcinogens and SEOM obtained from $PM_{2.5}$ and PM_{10}.

8. Acknowledgment

We would like to thank to Dr. Omar Amador-Muñoz and his staff for the SEOM of the San Agustin station and all support. The data of this chapter were obtained of the experimental work supported by Fondos Federales of the Dirección de Investigación of the Hospital Infantil de México Federico Gómez HIM/2010/013 and HIM/2011/008. Also the support of the Coordinación de Investigación of Universidad Simón Bolívar.

9. References

Agency for Toxic Substances and Disease Registry (ATSDR). (1995). Toxicological profile for polycyclic aromatic hydrocarbons. In: *Toxicity of Polycyclic Aromatic Hydrocarbons (PAHs)*. 02-01-11. http://www.atsdr.cdc.gov/toxprofiles/index.asp

Amador-Muñoz, O., Villalobos-Pietrini, R., Miranda, J., & Vera-Avila, L E. (2011). Organic compounds of $PM_{2.5}$ in Mexico Valley: spatial and temporal patterns, behavior and sources. *Science Total Environ*, 409, 8, 1453-1465.

Brzeznicki, S., Jakubowski, M., & Czerski, B. (1997). Elimination of 1-hydroxypyrene after human volunteer exposure to polycyclic aromatic hydrocarbons. *Int Arch Occup Environ Health*, 70, 4, 257-260.

Buckley, T J., & Lioy, P J. (1992). An examination of the time course from human dietary exposure to polycyclic aromatic hydrocarbons to urinary elimination of 1-hydroxypyrene. *Br J Ind Med*, 49, 2, 113-124.

Castaño-Vinyals, G., D´Errico, A., Malats, N., & Kogevinas, M. (2004). Biomarkers of exposure to polycyclic aromatic hydrocarbons from environmental air pollution. *Occup Environ Med*, 61, 4, e12.

Cecinato, A., Balducci, C., Nervegna, G., Pareti, S., Tagliacozzo, G., Brachetti, A. (2008). Year time modulation of n-alkanes, PAH, nitro-PAH and organic acids at Montelibretti Rome, Italy. *Polycyclic Aromat Compd*, 28, 500-517.

Committee on the Medical Effects of Air Pollutants, COMEAP. (2011). Polycyclic aromatic hydrocarbons (PAHs). In: *Air pollutants of relevance to health*. 02-01-11, <http://www.comeap.org.uk/component/content/article/28/107-polycyclic-aromatic-hydrocarbons-pahs-and-other-toxic-organic-compounds.html>

Denison, M., & Nagy, S. (2003). Activation of the aryl hydrocarbon receptor by structurally diverse exogenous and endogenous chemicals. *Annual Rev Pharmacol Toxicol., 43*, 309-334.

Dietrich, C., & Kaina, B. (2010). The aryl hydrocarbon receptor (AhR) in the regulation of cell-cell contact and tumor growth. *Carcinogenesis,* 31, 8, 1319-1328.

Dor, F., Dap, W., & Empereur-Bissonnet, P. (1999). Validity of biomarkers in environmental health studies: the case of PAHs and benzene. *Crit Rev Toxicol*, 29, 2, 129-168.

Duan, H., Jiang, Y., Zhang, H., & Wu, Y. (2010). MiR-320 and miR-494 affect cell cycles of primary murine bronchial epithelial cells exposed to benzo[a]pyrene. *Toxicol In Vitro*, 24, 3, 928-935.

Elovaara, E M J., Stockmann-Juvala, H., Luukkanen, L., Keski-Hynnila, H., Kostiainen, R., Pasanen, M., Pelkonen, O., & Vainio H. (2006). Polycyclic aromatic hydrocabon (PAH) metabolizing enzyme activities in human lung, and their inducibility by exposure to naphtalene, phenanthrenen, pyrene, chrysene, and benzo (a)pyrene as shown in the rat lung and liver. *Arch Toxicol, 81*, 3, 169-182.

Gachanja, A N. (2009). PAHs determination. Environmental applications, In: *Regional Synthesis Report on the Status of Pollution in the Western Indian Ocean Region,* 02-01-11, www.unep.org/NairobiConvention/docs

Gasiewicz, T H., & Collins, L. (2008). Expression and activity of aryl hydrocarbon receptor in development and cancer. *Crit Rev Euk Gene Exp,* 18, 4, 279-321.

Gerhard, S. (2005). Biomonitoring of inhaled complex mixtures-ambient air, diesel exhaust and cigarette smoke. *Exp Toxicol Path, 57*, Suppl 1, 75-110.

Irigaray, P., & Belpomme, D. (2010). Basic properties and molecular mechanisms of exogenous chemical carcinogens. *Carcinogenesis,* 2, *31*, 135-148.

Jardim, M J., Fry, R C., Jaspers, I., Dailey, L., & Diaz-Sanchez, D. (2009). Disruption of microRNAs expression in human airway cells by diesel exhaust particles is linked to tumorigenesis-associated pathways. *Env Health Persp*, 117, 11, 1745-1751.

Jiang, Y., Wu, Y., Greenlee, A R., Wu, J., Han, Z., Li, X., & Zhao, Y. (2011). MiR-106a-mediated malignant transformation of cells induced by anti-benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxide. *Toxicol Sci* 119, 1, 1, 50-60.

Joost, H M., van Delft, K M., Yvonne, C M., Marcel, H M., van Herwijnen, K., Brauers, J J., & Kleinjans C S. (2010). Time series analysis of benzo (a) pyrene-induced transcriptome changes suggest that a network of transcription factors regulates the effects on functional genes sets. *Toxicol Sci, 117*, 2, 381-392.

Kalscheuer, S., Zhang, X., Zeng, Y., Upadhyaya, P. (2008). Differential expression of microRNAs in early-stage neoplastic transformation in the lungs of F344 rats chronically treated with the tobacco carcinogen 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone. *Carcinogenesis*, 29, 12, 2394-2399.

Kim, V N. (2005). MicroRNA biogenesis: coordinated cropping and dicing. *Nature Rev Mol Cell Biol*, 6, 5, 376-385.

Kuhn, D E., Martin, M M., Feldman, D S., Terry, A V Jr., Nuovo, G J., Elton, T S. (2008). Experimental validation of miRNA targets. *Methods*, 44, 1, 47-54.

Lee, H Y., & Yang, M. (2008). Applications of CYP-450 expression for biomonitoring in environmental health. *Env Health Prev Med,* 13, 2, 84-93.

Lin, P., Tsai, W., Wu, M., Liao, Y., Chen, J., & Su, J. (2003). Overexpression of aryl hydrocarbon receptor in human lung carcinomas. *Toxicol Path,* 31, 1, 22-30.

Liu, L., Jiang, Y., Zhang, H., Greenlee, A R., Yu, R., & Yang, Q. (2010). MiR-22 functions as a micro-oncogene in transformed human bronchial epithelial cells induced by anti-benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxide. *Toxicol In vitro*, 24, 4, 1168-1175.

Lu, Y., Thomson, A G., Wong, H Y., Hammond, S M., & Hogan, B L. (2007). Transgenic over-expression of the microRNAs miR-17-92 cluster promotes proliferation and inhibits differentiation of lung epithelial progenitor cells. *Dev Biol*, 10, 2, 442-453.

Maliszewska-Kordybach, B. (1999). Sources, concentrations, fate and effects of Polycyclic Aromatic Hydrocarbons (PAHs) in the environment. Part A: PAHs in air. *Polish J Environ Studies*, 8, 3, 131-136.

Marley, C A., Gasiewicz, T A., Sime, P J., & Phipps, R P. (2005). The aryl hydrocarbon receptor is a regulator of cigarette smoke induction of the cyclooxygenase and prostaglandin pathways in human lung fibroblasts. *American J Physiol Lung Cell Mol Physiol,* 289, 3, 391-399.

McGrath, T., Wooten, J B., Chan, G., W., & Hajaligol, M R. (2007). Formation of polycyclic aromatic hydrocarbons from tobacco: The link between low temperature residual solid (char) and PAH formation. *Food Chem Toxicol*, 45, 6, 1039-1050.

Nana-Sinkam, S P., Hunter, M G., Nuovo, G J., Schmittgen, T D., Gelinas, R., Galas, D., & Marsh, C B. (2009). Integrating the microRNome into the study of lung disease. *Am J Respir Crit Care Med,* 179, 1, 4-10.

Nebert, D T., Okeys, A., & Gonzalez, F. (2004). Role of aryl hydrocarbon receptor-mediated induction of the CYP 1 enzymes in environmental toxicity and cancer. *J Biol Chem, 279*, 23, 23847-23850.

Nguyen, L., & Bradfield, C. (2008). The search of endogenous activators of the aryl hydrocarbon receptor. *Chem Res Toxicol,* 21, 1, 102-116.

Pavek, P., & Dvorak, Z. (2008). Xenobiotic-induced transcriptional regulation of xenobiotic metabolizing enzymes of the cytochromeP450 super family in human extrahepatic tissues. *Current Drug Metabolism,* 9, 2, 129-143.

Rajput, N., & Lakhani, A. (2010). Measurement of polycyclic aromatic hydrocarbons in an urban atmosphere of Agra, India. *Atmósfera*, 23, 2, 165-183.

Schembri, F., Sridhay, S., Perdomo, C., Gustafson, A M., Zhang, X., Ergun, A., Lu, J., Liu, G., Zhang, X., Bowers, J., Vaziri, C., Ott, K., Sensinger, K., Collins, J J., Brody, J S., Gatts, R., Lenburg M E., & Spira A. (2009). MicroRNAs as modulators of smoking-induced gene expression changes in human airway epithelium. *Proc Natl Acad Sci USA*, 106, 7, 2319-2324.

Shen, Y-L., Jiang, Y-G., Greenlee, A R., Zhou, L-L., Liu, L-H. (2009). MicroRNA expression profiles and miR-10[a] target in anti-benzo[a]pyrene-*trans*-7,8-diol-9,10-epoxide-transformed human 16HBE cells. *Biomed Environ Sci*, 22, 1, 4-21.

Shimada, T. (2006). Xenobiotic-metabolizing enzymes involved in activation and detoxification of carcinogenic polycyclic aromatic hydrocarbons. *Drug Metabolism Pharm, 21,* 4, 257-276.

Shimada, T., Fujii-Kuriyama, Y. (2004). Metabolic activation of polycyclic aromatic hydrocarbons to carcinogens by cytochromes P450 1A1 and 1B1. *Cancer Science, 95*, 1, 1-6.

Spira, A., Beane, J E., Shah, V., Steiling, K., Liu, G., Schembri, F., Gilman, S., Dumas, Y M., Calner, P., Sebastiani, P., Sridhar, S., Beamis, J., Lamb, C., Anderson, T., Gerry, N., Keane, J., Lenburg, M E., & Brody, J S. (2007). Airway epithelial gene expression in the diagnostic evaluation of smokers with suspect lung cancer. *Nat Med*, 13, 3, 361-366.

Spivack, S., Fasco, H G., & Kaminsky, M L. (2003). Phase I and II Carcinogen Metabolism Gene Expression in Human Lung Tissue and Tumors. *Clinical Cancer Res, 9*, 16 Pt 1, 6002-6011.

Taioli, E. (2008). Gene-Environment interaction in tobacco-related cancers. *Carcinogenesis,* 29, 8, 1467-1474.

Tomankova, T., Petrek, M., & Kriegova, E. (2010). Involvement of microRNAs in physiological and pathological processes in the lung. *Resp Res*, 11, 159, 1-10.

United States for Environmental Protection Agency, USEPA. (1993). Provisional guidance for quantitative risk assessment of polycyclic aromatic hydrocarbons. 02-01-11. EPA/600/R-93/089

Villalobos-Pietrini, R., Amador-Muñoz, O., Waliszewski, S., Hernandez-Mena, L, Munive-Colin, Z., Gómez-Arroyo, S, Bravo-Carrera J L., & Frías-Villegas, A. (2006). Mutagenicity and polycyclic aromatic hydrocarbons associated with extractable organic matter from airborne particles ≤ 10 μm in southwest Mexico City. *Atmospheric Env*, 40, 5845-5857.

Wang, F., Zhang, R., & Hankinson, O. (2006). Identifying target genes of the aryl hydrocarbon receptor nuclear translocator (Arnt) using DNA microarray analysis. *Biol Chem,* 387, 9, 1215-1218.

Wang, G., Huang, L., Zhao, X., Niu, H., & Dai, Z. (2006). Aliphatic and polycyclic aromatic of atmospheric aerosols in five locations of Nanjing urban area, China. *Atmos Res*, 81, 1, 54-66.

Wang, X Y., Li, Q B., Luo, Y M., Ding, Q., Xi, L M., Ma, J M., Li, Y., Liu, Y P., & Cheng, C L. (2010). Characteristics and sources of atmospheric polycyclic aromatic hydrocarbons (PAHs) in Shangai, China. *Environ Monit Assess*, 165, 1-4, 295-305.

Weiling, X., & Warshawsky, D. (2005). Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: A review. *Toxicol Appl Pharmacol, 206*, 1, 73-93.

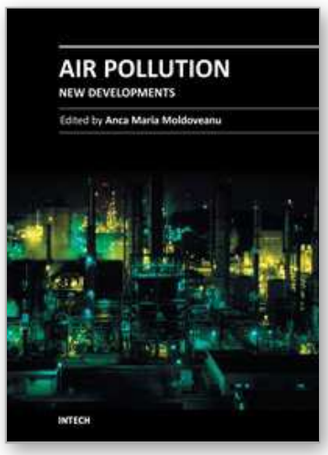
Wichmann, H E. (2007). Diesel exhaust particles. *Inhal Tox*, 19, suppl1, 241-244.

Williams, A E., Moschos, S A., Perry, M M., Barnes, P J., Lindsay, M A. (2007). Maternally imprinted microRNAs are differentially expressed during mouse and human lung development. *Dev Dyn*, 236, 2, 572-580.

Wincent, E A., Lueche, S., Glatt, H., Bergman, J., Cresenzi, C., Rannug, A., & Rannug, U. (2009). The suggested physiologic aryl hydrocarbon receptor activator and cytochrome P4501 substrate 6- Formylindolo[3,2-b]carbazole is present in humans. *J Biol Chem,* 284, 5, 2690-2696.

Working Group of Polycyclic Aromatic Hydrocarbons. (2001). Ambient Air Pollution by PAH´s Position Paper, In: *Office for Official Publications of the European Communities*, 02-01-11, <http://ec.europa.eu/environment/air/pdf>

Zhang, Y., & Tao, S. (2009). Global atmospheric emission inventory of polycyclic aromatic hydrocarbons. *Atmosph Environ*, 43, 4, 812-819.



Air Pollution - New Developments

Edited by Prof. Anca Moldoveanu

ISBN 978-953-307-527-3
Hard cover, 324 pages
Publisher InTech
Published online 06, September, 2011
Published in print edition September, 2011

Today, an important issue is environmental pollution, especially air pollution. Due to pollutants present in air, human health as well as animal health and vegetation may suffer. The book can be divided in two parts. The first half presents how the environmental modifications induced by air pollution can have an impact on human health by inducing modifications in different organs and systems and leading to human pathology. This part also presents how environmental modifications induced by air pollution can influence human health during pregnancy. The second half of the book presents the influence of environmental pollution on animal health and vegetation and how this impact can be assessed (the use of the micronucleus tests on TRADESCANTIA to evaluate the genotoxic effects of air pollution, the use of transplanted lichen PSEUDEVERNIA FURFURACEA for biomonitoring the presence of heavy metals, the monitoring of epiphytic lichen biodiversity to detect environmental quality and air pollution, etc). The book is recommended to professionals interested in health and environmental issues.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Francisco Arenas-Huertero, Elisa Apátiga-Vega, Gabriela Miguel-Pérez, David Villeda-Cuevas and Jimena Trillo-Tinoco (2011). Molecular Markers Associated with the Biological Response to Aromatic Hydrocarbons from Urban Air in Humans, Air Pollution - New Developments, Prof. Anca Moldoveanu (Ed.), ISBN: 978-953-307-527-3, InTech, Available from: http://www.intechopen.com/books/air-pollution-new-developments/molecular-markers-associated-with-the-biological-response-to-aromatic-hydrocarbons-from-urban-air-in

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

© 2011 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen