Latest News on Occupational Health

Chapter 2

Application of Novel Molecular Biomarkers in the Occupational Medicine

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Abstract

The biological monitoring represents a useful tool for the health risk assessment of workers exposure in the occupational medicine. Exposure to dangerous substances, particularly to those that are carcinogenic for humans, represents a significant health hazard to workers who may develop malignant tumours following persistent exposure to toxic agents with carcinogenic properties. The use of molecular biomarkers in the occupational field is fundamental not only to evaluate the potential risk associated to specific toxicants but also to prevent the onset of disease. Historically, the classification of biomarkers used in the biological monitoring consists of three categories: dose/exposure biomarkers; effect biomarkers and susceptibility biomarkers. However such indicators, even though are essential in the experimental campaigns, do not provide sufficient indications for cancer prevention. In the last decade, with the development of high-throughput technologies and the progressing knowledge in molecular and clinical medicine, it became possible to identify a new generation of indicators, namely epigenetic biomarkers, that might integrate the traditional ones. These novel markers reflect

the effects of the environmental, occupational and individual lifestyle exposure, being able to modify the genome without changing the DNA nucleotide sequence. In this chapter we discuss some relevant studies on the application of novel epigenetic biomarkers in the occupational field that can make workers susceptible to cancer development.

Keywords: Cancer; DNA Methylation; Effect Biomarker; Epigenetic Biomarker; MicroRNA; Occupational Exposure; Risk Assessment.

1. Introduction

Exposure to hazardous substances, particularly to those that are carcinogenic for humans, represents a significant health hazard to workers who could develop tumours following chronic exposure to such agents. The complex carcinogenesis mechanism is determined by the accumulation and interaction of genetic and epigenetic abnormalities that affect the structure and function of the genome resulting in dysregulated gene expression and function. The use of molecular biomarkers in the occupational field is fundamental not only to evaluate the potential risk associated to specific toxicants but also to prevent the onset of disease. The observation that epigenetic changes are reversible makes them an attractive target for disease prevention and treatment suggesting their use in preventive occupational medicine.

Personal lyfe style and habits, diet, drug assumption, alcohol consumption as well as the variable environmental and occupational conditions to which individuals are exposed represent the complexity of human daily exposure factors determining the induced health effects. These factors are key issues that may affect “the epigenetic modifications” on human DNA. Epigenetics covers heritable changes in the functions of genes that occur without direct alteration in the DNA sequence itself [1].

The majority of epigenetic regulation generally occurs on DNA and chromatin, where different modifications may appear. These include DNA methylation, histone post-transcriptional modification i.e. ubiquitination, acethylation, phosphorylation, resulting in chromatin reorganization. Despite the DNA sequence remains unchanged a reshape of the chromatin stucture and conformation may occur, contributing to the modulation of gene expression. The epigenetic influence on DNA does not subvert the genetic code but may result in activation or silencing of specific genes as consequence of different stimuli. In summary, differently from gene mutations, which are fixed and heritable along generation, the epigenetic biomarkers are reversible and may alter gene expression in a heritable manner [2].

To date, three main epigenetic molecular mechanisms have been considered relevant: 1) DNA methylation, 2) nucleosome modification with reshape of chromatine conformation; 3) microRNA synthesis (**Figure 1**). Several clinical studies showed that epigenetic biomarkers are useful to prevent and cure several diseases with particular attention to cancer.

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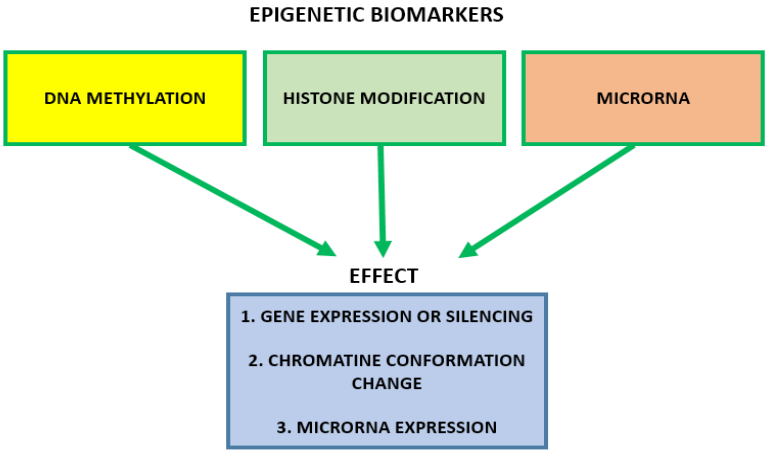

Figure 1: Main epigenetic molecular modifications.

These novel biomarkers differ from the traditional indicators used in the biomonitoring of exposed workers. The latter comprises three types of markers: 1) internal dose, 2) early effect 3) susceptibility. The first group (exposure-dose biomarkers) detects the amount of dangerous substance absorbed by the subject allowing the measure of toxicant levels excreted in biological fluids (i.e. urine). The second group (biomarkers of early effect) reveals the DNA damage caused by genotoxicity and chromosomal aberrations in the cell of the individual. The third group (susceptibility biomarkers), analyzes the variation of polymorphic genes in the population, providing a variable response to the chemical insults which is related to the genetic inheritance and exposure type. Nowadays a fourth group of biomarkers, namely the epigenetic biomarkers, has been taken in cosideration particularly in the medicine field. Such biomarkers, emerging in the last twenty years, reflect DNA modifications caused by exogenous exposures, meaning that gene expression or silencing does not depend on the nucleotide sequence of the gene.

Mainly used in the clinical medicine, epigenetics is becoming also a crucial component of the occupational scenario where modifications reflect the effect of exposure on the human genome. In particular, they contribute i) to bridge the gap between genetic background and personal exposure; ii) to clarify the molecular mechanisms responsible for disease development, with particular attention to cancer [3]. Epigenetic modifications probably occur at a very early stage in cancer development, and they are essential determinants in cancer progression [1]. Therefore epigenetic biomarkers could represent promising markers for early detection, disease monitoring, prognosis, and risk assessment of cancer induction to be used in occupational medicine.

2. Bibliography Search Method

A bibliographic search strategy has been carried out to select scientific journal articles inherent to the epigenetic field. Papers on epigenetic biomarkers were retrieved from PubMed database (pubmed.ncbi.nlm.nih.gov).

A list of search terms has been used as key words to select the articles. As an example "occupational exposure and carcinogens" or "occupational medicine and epigenetic biomarkers" and "epigenetic modification" have been used to identify the studies wherein the risk of occupational exposure to carcinogens correlates with the presence of epigenetic modifications. The parametres to select the most interesting and valuable studies, based on the term "exposure" "epigenetics" and "carcinogenic compound" have been searched looking for the journal articles published in the last 10-15 years. The inclusion criteria were: journal articles written in English language on "epigenetics" and associated to the term "occupational" or "workplace", "epigenetic DNA modifications" and "microRNA".

The date limits that were set for choosing the most relevant articles were approximately from 2000 to 2020, except for few seminal or experimental papers which have been published before 2000 but whose relevance is widely recognized by experts in the field.

The following key words or search strings in the Pubmed database were:

• occupational risk and cancer and epigenetics

• occupational exposure and carginogenic substances and DNA methylation

• epigenetics and microRNAs and biomarkers of effect

All the selected articles were based on the relevance of the objective of the study. A total number of fifty-six papers have been retrieved. Some of these concerned the exposure to specific carcinogenic substances i.e. benzene, arsenic, asbestos, chromium, volatile organic compounds.

3. Epigenetic Biomarkers

It has been stated that hypermethylation on the CpG sequence determines gene silencing with mRNA suppression. On the other hand, hypomethylation of CpG sequence activates RNA transcription and gene expression. The methyl groups are added by S-adenosylmethionine on Carbon 5,2, of cytosine preceeding guanine (CpG island) (**Figure 2**).

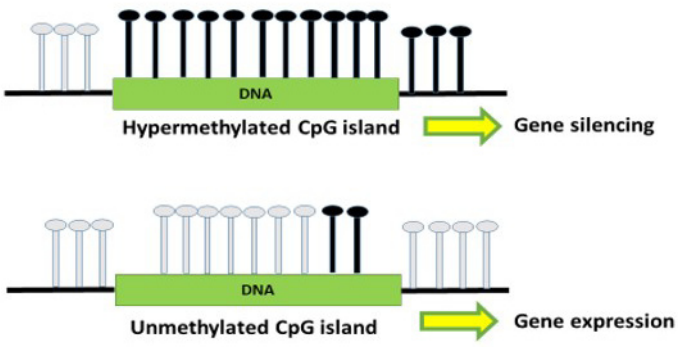

Figure 2: DNA methylation mechanism of action.

The DNA methylation generally results in the silencing of specific genes as well as in non-coding introns and ripetitive elements carried out by DNA methyl-transferase (DNMTs) (**Figure 2**). The process is dynamic and is counterbalanced by demethylation, which is controlled by the methylcytosine dioxygenase, Ten Eleven Translocation (TET), which progressively oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) [4]. The DNA methylation can recruit proteins binding CpG sequence such as: methyl CpG binding domain proteins (MBD), histone deacetylase (HDAC) and histone methyltransferase (HMT). Those proteins coordinate modifications of the surrounding chromatine. Different epigenetic profiles have been identified in association to several disease, contributing to the understanding of molecular mechanisms that might be used as potential and functional biomarkers. Histones also undergo addition of chemical groups including acethylation, methylation, phosphorylation, sumoilation and ubiquitination. Addition of the chemical groups contribute to the modulation of chromatin and nucleosome.

It has been confirmed by scientific evidence that carcinogenesis site-specific hypermethylation is associated to a global DNA hypomethylation, where the absence of methylation is a reliable biomarker of the oncogenesis process [5]. TET genes, and especially TET2, are frequently mutated in various cancers, but how the TET proteins contribute to prevent the onset and maintenance of these malignancies is largely unknown. Alterations in TET protein found in malignant tumours, suggests that modifications occurring in the dynamic mehtylation-demethylation process may be the first step of carcinogenesis [6].

3.1. DNA methylation

Not only the genes encoding proteins but also the non-coding RNA, with inibitory function, undergo gene silencing due to DNA methylation in tumoural cells, while other non-coding RNAs can face epigenetic modification, favoring the tumour onset [7-8].

The identification of epigentic profiles is possible employing different innovative techniques such as the bisulfite method associated to sequencing of the whole genome. The aim is to identify and compare normal DNA methylation profiles (i.e.reference profile) and aberrant DNA methylation profile (modified profile). The bisulfite technique is based on chemical deamination of unmethylated cytosines, modified in uracil, while the methylated cytosines remain unmodified. This technique allows to achieve useful information on DNA methylation pattern at the level of single nucleotide. Following cytosine conversion into uracil and sequencing it is possible to identify all the methylated DNA regions in the biological sample [9].

Another routinary method used to determine DNA methylation on the entire genome is the DNA microarray immunoprecipitation or sequencing (MeDIP-chip/seq) that makes use of anti-methylcytosine antibodies to immunoprecipitate DNA, enriched in highly methylated

CpG islands with fragments of 150-300 bp. The immunoprecipitation is followed by sequencing analysis. However this technique shows some limitations, depending on the amount of CpG present in the site recognized by antibodies. It also shows reduced resolution in comparison to the bisulfite technology, causing artifacts or unreliable results [10]. Other semi-quantitative methods include conventional and colorimetric enzyme-based immunoassays, where the quantification of 5mC and 5hmC is achieved using specific monoclonal antibodies followed by addition of secondary horseradish-peroxidase-conjugated-antibody (**Figure 3**). The signal may also be amplified using the streptavidin-biotin method. This assay, although not sensitive as the bisulfite pyrosequencing method, is anyway applicable to human samples resulting in differential profiles of methylated DNA in malignant transformation compared to the non malignant one [11].

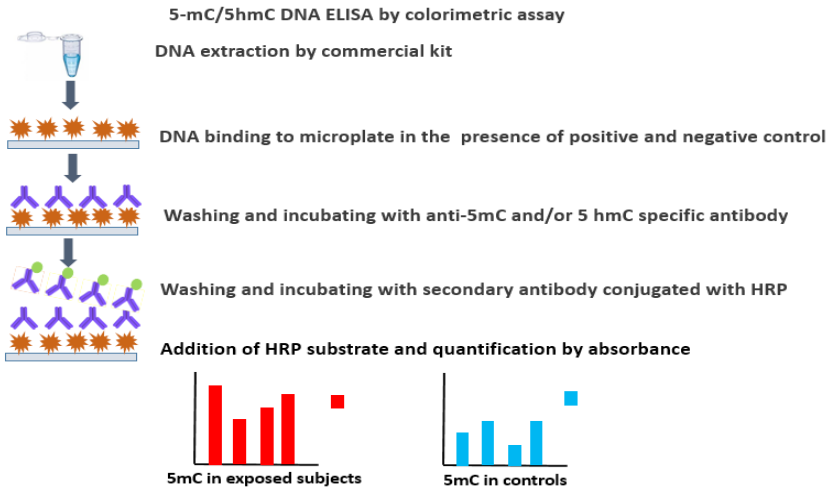

Figure 3: Colorimetric assay used to assess DNA methylation.

Considering the human cancer, two categories of changes in DNA methylation typical of malignancy have been observed with respect to the normal tissue; ì) hypomethylation of specific genome regions; ìì) de novo hypermethylation on CpG islands. Such epigenetic profile is considered a biomarker of early effect, typical of carcinogenesis [12].

3.2. microRNA

MicroRNAs, abbreviated as miRNAs, recently emerged as further category of epigenetic biomarkers. These are small, non-coding, single-stranded RNAs of approximately 20-22 nucleotides in lenght, able to influence target gene expression. miRNAs are transcribed from DNA sequences into long precursors to undergo a final processing and become mature miRNAs. In several cases miRNAs may interact with the 3′ untranslated regions (UTR) of the target messenger RNA to suppress the gene expression; they also affect the coding sequence as well as the gene promoters [13] (**Figure 4**). In the last twenty years, aberrant expression of miRNAs has been intensively recognized in the pathogenesis of diseases, including cancer, identifying these small molecules as new source of prognostic and diagnostic biomarkers with large applicability to the clinical medicine [14]. They emerged as promising candidate bio-

markers since are abundant in circulation, highly stable in the biological fluids and may yield diagnostic signatures. MiRNAs have been detected in several diseases; from pathogenesis of hearing disorders, cardiovascular and neurological disease i.e. Alzheimer's as well as in the carcinogenesis of different organs including liver, lung, breast, brain and intestine [15-17].

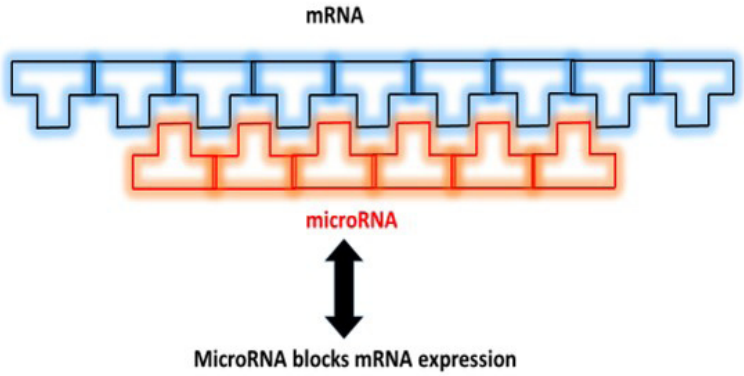

Figure 4: MicroRNA-mediated gene silencing.

The interest in miRNAs as epigenetic regulators has recently increased also in the occupational and environmental medicine since they can be employed as useful indicators of exposure to toxicants and carcinogenic agents. For instance, miRNAs are emerging as good candidates of exposure to toxic substances such as volatile organic compounds (VOCs) used for instance in painting activities [18]. A novel study performed on the occupational setting involved a group of workers exposed to solvents compared to controls. Circulating miRNAs found in the plasma of each subject have been analyzed in both groups. Fifty-six differentially expressed miRNAs were identified at a statistically significant level. In particular, four miRNAs belonging to a small subset strongly related to VOCs and relative metabolites i.e. miR-589-5p, miR-941, miR-146b-3p and miR-27a-3 seem promising biomarkers to predict the health risk caused by mild exposure to the occupational VOCs. The authors conclude miRNA expression may represent an early and predictive tool for identification of exposure-related disease particularly when multiple toxicants, generating oxidative stress and inflammation processes, are contemporary used by workers [19, 20]. It is widely known miRNAs show many advantages in terms of novel biomarkers, both biological and technical. These are:

1) easy extraction from plasma or other biological fluids with reliable commercial extraction kits;

2) robust and consistent expression by several molecular techniques i.e. Next Generation Sequencing (NGS) analysis; quantitative PCR; human microRNA array. All these techniques are followed by bioinformatic analysis resulting in the identification of differential miRNA profiles among subjects:

3) identification of miRNA signatures with significant difference between cancerous and matched non-cancerous tissue.

In that respect, many studies pointed out that in the event of exposure to hazardous carcinogenic chemicals at workplace, individual alterations in miRNA profile may occur, recognizing the use of circulating miRNAs, from full blood, serum or plasma, as useful and predicted indicators of risk evaluation.

4. Occupational Medicine Studies Based on Epigenetic Biomarkers

Historically, epigenetic biomarkers have been discovered and used in the clinical medicine, with particular attention to malignant tumours. Nowdays, after years of research, it seems possible to transfer this know-how about cancer prediction and prevention in humans to include the field of occupational medicine.

The aim is to identify categories or sub-populations at high risk of exposure to be addressed to prevention and follow-up of early occupational disease. The detection of epigenetic modifications as potential biomarkers of occupational cancerogenesis is possible because of their stability, frequency and non-invasive identification methods. However, the major difficulty derives from the limitation of specificity and sensitivity of some malignant tumours which, being silent for years, might escape the immune system response of the individual using different strategies [21]. In the following section we identified some significant studies where the exposure to carcinogenic substances may induce malignant tumours after long-time exposure. Among these we mention the following dangerous substances that we believe are worthy of note: benzene, arsenic, asbestos and exavalent chromium. Allt these compounds have been classified by IARC (International Agency for Research on Cancer) in the first group of carcinogenic substances to human being.

4.1. Benzene

Benzene is a worldwide carcinogenic pollutant mainly present in the environment as well as in the workplace. It is a volatile organic compound used in several human activities, such as those performed by petrol station attendants, traffic wardens, petrochemical plant workers and painters. For this dangerous substance there is no safety exposure limit. However the American Conference of Governmental Industrial Hygienists (ACGIH) reports benzene concentrations in the environmental and occupational context similar to those present in the occupational setting (1–1000 $\mu g/m^3$) demonstrating it is ubiquitous. The most recent exposure limits for benzene in the urban zone has been established at 5 $\mu g/m^3$. Although the benzene mechanism leading to high toxicity is not clear, the danger of this compound is strictly dependent on the individual metabolism, activating the production of reactive intermediates that are hazardous for the organism. Benzene detoxification, like for all the other chemicals, depends on the individual susceptibility to the compound. Several experimental and epidemiology evidence demonstrated that chronic exposure to benzene is associated to an increase of hema-

tological disease, including aplastic anemia and acute myeloid leukemia [22]. Other studies indicated that exposure to benzene induces loss of genomic methylation (global DNA hypomethylation) in human lymphoblastoid TK6 cells [23]. Similar epigenetic alterations were found in hematopoietic malignancies, particularly in patients with acute myeloid leukemia where other genetic alterations may occur [24]. On the other hand, further studies indicate that benzene induces DNA hypermethylation of the tumor suppressor genes p15INK4b and p16INK4a in benzene exposed workers [25].

In a cross-sectional study on a petrochemical plant workers exposed at low benzene levels (<1ppm, a value lower than the limit established for the occupational exposure) it has been observed that benzene could induce DNA hypermethylation of two oncosuppressor genes (p15INK4b and p14ARF) mainly in exposed subjects than in controls. This observation has been confirmed by previous studies where a significant association between hypermethylation of p15INK4b gene and increased environmental benzene levels has been confirmed in a group of petrol station attendants and traffic wardens exposed to very low benzene doses [26].

4.2. Arsenic

Inorganic arsenic is a dangerous carcinogenic substance for the human health. Evidence suggests that people chronically exposed to inorganic arsenic are at risk of developing cancer or cardiovascular, neurological, and metabolic diseases. Arsenic may induce cancer in specific organs such as in lung, skin, bladder, liver, kidney and pancreas by inhalation or oral consumption of contaminated water [27].

The occupational exposure to inorganic arsenic is a serious risk factor to the human health. Some body districts particularly in lung, skin and bladder are associated to arsenic exposure through inhalation or by drinkable water. In the occupational field arsenic may be present also in food supply and animal husbandry chain, facilitating the assumption of this toxic compound by the population. Epidemiological literature reports arsenic neurotoxicity in children and adults with emphasis on the cognitive dysfunction, including learning, memory deficits and mood disorders [28].

Although the mechanism of carcinogenesis induced by arsenic is not clear, such element, despite it is not strongly mutagenic, is able to induce epigenetic silencing on the oncosuppressor gene p16INK4 by DNA hypermethylation [29]. Previous work has demonstrated that inorganic arsenic can alter miRNA expression patterns in *in vitro* and *in vivo* models of arsenic-induced carcinogenesis. Both up and downregulated miRNAs have been associated with cancer, acting either as oncogenes, tumour suppressors, or both [30]. miR-182-5p suppression was shown to contribute to hypoxia-inducible factor HIF2α overexpression in response to arsenite exposure, suggesting that aberrant overexpression of HIF2α via miRNA dysregulation is involved in arsenic-induced carcinogenesis [31]. After arsenic exposure, analysis of

miR-200 family members, specifically miR-205, indicated that deregulated miRNAs could be potential biomarkers for arsenic exposure and be used as diagnostic markers for the onset of early urothelial carcinoma [32]. Several studies have focused on miRNAs as promoters of the apoptosis induced by arsenic trioxide, which is commonly used in the treatment of acute promyelocytic leukemia, mainly supporting the hypothesis that miRNAs may play a mediatory role in eliciting the multitarget action of this compound [33].

Ruiz-Vera and collaborators [34] demonstrated an alteration in the expression levels of two miRNAs (miR-155 and miR-126) associated with cardiovascular disease in women in Mexico exposed to inorganic arsenic via drinking water. Similarly, high arsenic exposure was associated with altered profiles of circulating miRNAs in plasma of healthy subjects from Mexico and four of the identified miRNAs (miR-423-5p, miR-142-5p, and miR-454-5p) appeared to be linked to cardiometabolic disease risk [35]. In a further study that investigated the health impact from prenatal exposure, ranging up to 236.0 μg arsenic/L in drinking water in Mexico, researchers found a set of differentially expressed miRNAs and mRNAs that were implicated in the innate and adaptive immune response [36]. The levels of miR-21 were upregulated in individuals from the highly arsenic-contaminated district of West Bengal where, within the exposed group, miR-21 expression levels were higher in the individuals with skin lesions when compared with the individuals without skin lesions [37].

4.3. Asbestsos

Asbestos is a natural mineral fibrous material with known carcinogenic properties widely used in the past particularly in the production of building material. It includes six mineral fibers: five amphiboles fibers (crocidolite, actinolite, tremolite, anthophyllite, and amosite) and one serpentine fiber (chrysotile).

In Italy the extraction and use of asbestos was banned in 1992 but there are many countries where asbestos is still being used [38].

It is widely known asbestos is the major cause of malignant mesothelioma and it has been indisputably considered associated with the fiber exposure [39]. Scientific evidence demonstrated association of asbestos exposure with the pleural and peritoneal malignant mesothelioma, with a latency of approximately forty years following exposure and from the initial development of disease. The importance of epidemiologic surveillance has been confirmed as effective tool for public health and welfare policies.

Malignant pleural mesothelioma (MPM) has been known as very resistant tumour to therapy compared to melanoma and non-small cell lung cancer [40]. The epidemiology surveillance is a fundamental tool to monitor the health effects of patients with conclamed disease and to assess the efficacy of prevention in individuals exposed to asbestos but disease-free. Nu-

merous studies demonstrated that clastogen and cytotoxic mechanisms following exposure to asbestos fibers induce strong inflammation caused by frustrated phagocytosis of macrophages resulting in persistent oxidative stress. These are important factors that may contribute to the mesothelioma growth [41]. Furthermore patients undergoing surgical resection of MPM might have relapse making difficult further surgical operations.

To date, the need of prognostic and reliable biomarkers of MPM is auspicable, particulary for the tumour-free exposed subjects in order to prevent the onset of malignant disease, but also for patients with stable disease since the available medical treatment does not ensure their survival.

The traditional biomarkers used in mesothelioma are proteins such as mesothelin (MSLN), osteopontin, fibulin and high mobility group 1 proteins (HMGB1). However, none of them seem to be reliable indicators of the disease. More recently, with the advent of epigenetics, a novel generation of biomarkers have been studied and are being used by researchers to prevent such fatal disease for both affected and tumour-free exposed subjects. In this context, the unique properties of epigenetic biomarkers, particularly regarding DNA methylation and miRNA expression might represent promising prognostic and diagnostic tools for this malignant disease.

One of the most studied gene of MPM is mesothelin encoding the mesothelin-related peptide (SMRP). Using the bisulfite pyrosequencing it has been observed that methylation of the MSLN promoter was significantly higher in the normal pleural tissue than in the tumour. However SMRP, despite being the most studied and frequently used biomarkers in MPM, showed poor sensitivity. The authors conclude that MSLN is normally methylated in the pleura and that the methylation is lost in most tumours, underlining the need of additional biomarker targets that will resolve the poor sensitivity of the SMRP assay and allow implementation of screening among exposed populations [42].

In another study, two epigenetic regulated markers miR-126, and methylated thrombomodulin promoter (Met-TM) were combined with SMRP and evaluated as potential strategy to detect malignant mesothelioma at an early stage. Forty-five malignant mesothelioma patients, twenty patients with non small cell lung carcinoma (NSCLC) and fifty-six healthy controls were recruited. The circulating miR-126 detected in the serum by quantitative real-time PCR (qRT-PCR) was found low-expressed in both malignancies, significantly differentiating mesothelioma patients from healthy controls and NSCLC from mesothelioma patients, but could not discriminate the NSCLC patients from the control subjects. In this work the authors conclude that miR-126 is a sensitive disease marker but with lack of specificity, suggesting to use this biomarker in combination with other cancer-specific indicators such as mesothelin [43].

MiR-103 was reported as significantly downregulated biomarker in the blood cell fraction of twenty-three patients with MPM, compared to seventeen subjects previously exposed to asbestos and twenty-five healthy controls from the population. Q-RT-PCR was used for validation of miR-103 in patients, asbestos-exposed subjects, and healthy unexposed controls. The sensitivity and specificity shown by miR-103 to distinguish mesothelioma patients from healthy controls was 78% and 76%. However the miRNA found by researchers cannot be proposed as absolute biomarker and should be investigated further in future prospective studies [44].

A further analysis on miRNA expression has been carried out in the serum of fourteen MPM patients and ten control subjects with non-neoplastic pleural effusions. Five miRNAs (miR-101, miR-25, miR-26b, miR-335, and miR-433) were upregulated in contrast to two miRNA (miR-191, miR-223) that were downregulated and miR-29a and miR-516 were expressed exclusively in MPM patients. The authors proposed a different miRNA signature among the two groups characterized by different miRNA combinations but the results were judged not completely reliable due to the small sample size [45].

In another study 163 cases of MPM and 137 healthy controls have been considered to compare the DNA methylation profiles with particular attention to the CpG islands and the differentially methylated regions (DMRs). Most of the difference was observed in the genes involved in the deregulation of neutrophils of the native immune system and in the signalling pathway of the immune response. The authors inferred that difference in the DNA methylation profiles of circulating lymphocytes might help to distinguish MPM patients from the healthy exposed controls. Such difference might be exploited to elaborate a model of risk assessment of MPM in the asbestos exposed subjects [46].

4.4. Exavalent chromium

Further studies regarding the epigenetic modifications of DNA induced by occupational exposure to carcinogenic substances in the occupational field are related to chromium exavalent Cr(VI). This is a mineral element with strong genotoxic and carcinogenic properties. The limit value recognized at European level in the occupational exposure is 0,005 mg/m^3 (TWA 8h) (Directive 2019/130/EU).

Chromium inhalation has been associated to the increase of lung cancer in various productive sectors such as manufacture of steel, end alloy planting, dyes and pigments, painting, electroplating and metallurgy where the exposure may exceed 100 mg/m^3. The major mechanism of chromium-induced cytotoxicity is genotoxic since chromium, due to its various oxidation state, can induce oxidative stress, DNA strand breaks, DNA-protein crosslinks and production of DNA adducts. The mechanism of action through which Cr(VI) induces malignant transformation, might depend on epigenetic changes induced by genomic, chromosomal

instability and microsatellites (MSI), caused by the loss or alteration of the DNA mismatch repair genes (MMR). An in vitro study showed that a low dose of Cr(VI) exposure could induce mesenchymal epithelial transition and reinforce the invasion in the process of carcinogenesis of pulmonary epithelial cells [47]. The presence of methylation of p16 gene was investigated, by methylation-specific PCR method and by immunochemistry, on thirty chromate lung cancer samples and thirtyeight non-chromate lung cancers reporting that one third of patients affected by chromate lung cancers had methylation of p16. The authors indicated that chromate carcinogenesis may be due to both genetic and epigenetic alterations [48]. A further study demonstrated that chromium exposure increased the methylation level of the gene promoter of MLH1.The inactivation of hMLH1 expression strongly correlated with the microsatellite high instability phenotype in chromate lung cancer. The genetic instability of chromate lung cancer is due to the repression of hMLH1 protein. [49]. A recent study demonstrated that chromium exposure increased the methylation level of the gene promoter of MLH1 in the healthy tissue and mainly in the tumour tissue demostrating a positive correlation between the exposure time and the level of methylation in the pulmonary tissue. The results suggest epigenetic silencing of gene MLH1 expression, in response to Cr(VI) exposure is able to block the mismatch repair (MMR) making cells more susceptible to the transformation, being not capable to eliminate the damage induced by Cr(VI) exposure through apoptosis [50].

In a further study, published in 2020 on chromate exposed workers, the authors investigated the miRNA expression profile by using the microarray technology. Forty-five significant and differentially expressed miRNAs in Cr(VI)-exposed workers have been identified. In particular the results showed upregulation of twelve miRNAs and downregulation of other thirty-three miRNAs. In particular the analysis confirmed downregulation of a specific miRNAs group including miR-19a-3p, miR-19b-3p, and miR-142-3p and upregulation of miR-590-3p and miR-941. All the selected miRNAs have been isolated in the plasma of workers occupationally exposed to Cr(VI). The multiple linear regression analysis allowed to identify: Cr(VI) level in urine, exposure duration and age as risk factors affecting miRNA expression. In particular the downregulation of plasma miR-19a-3p in exposed workers negatively correlated with urinary Cr(VI), meaning that its concentration might be proposed as good indicator of short-term exposure to chromium. This study is promising since it might overlap the results obtained by the use of both epigenetic and exposure biomarkers [51].

5. Conclusion

The term “epigenetics” was introduced in 1942 by the embryologist Conrad Waddington who defined it as “the complex of developmental processes between the genotype and phenotype” [52]. Epigenetics explains how the same genotype can produce different phenotypes, due to the variety of external stimuli to which the individuals are exposed. Forty years ago, the discovery of global DNA hypomethylation in human cancer and the identification of CpG is-

-land promoter hypermethylation of oncosuppressor genes opened the door to the “novel epigenetic era” leading to the development of the “novel epigenetic markers” [11].

The recent advance in technologies using a multidisciplinary approach where molecular genetics, cell biology, toxicology, biochemistry, statistics and epidemiology are integrated reciprocally, are now making it possible to obtain complete and specific DNA methylome, histonome and non-coding RNA transcriptome profiles from any individual, affecting DNA conformation and gene expression. Such modifications, despite reversible and not necessarily inheritable, can be induced by exposure to a plethora of environmental and occupational factors, with the power to shift the balance between health and disease.

Cancer is the second leading cause of death in wealthy countries [53]. The use of non invasive techniques for prognosis and diagnosis of cancer represents the prior objective of the clinical medicine.The aberrations found in the pattern of DNA methylation have been recognized as typical of malignant tumours and might be exploitable to identify specific tailored therapies. During cancer onset a characteristic profile, represented by hypometylation of some DNA regions and hypermethylation on GpG islands of specific gene promoters, allow to classify these loci as early biomarkers of effect of incipient malignancy. A further main modification is represented by hypermethylation of the CpG islands of oncosuppressor genes, resulting in transcriptional silencing. Also miRNAs are good candidates as prognostic factors in cancer disease, where an abnormal expression pattern is clearly distinguishable from the normal one. MiRNAs identify aberrant signatures found in cancer disease associated with tumour diagnosis, staging, progression, prognosis and response to treatment [54]. Differential miRNA expression has been applied also to the occupational setting, where specific profiles in controls and exposed subjects might distinguish between high and low individual susceptibility to dangerous substances present in the workplace. The identification of early epigenetic alterations in exposed workers might be used as prognostic tools applicable to disease prevention.

In summary, epigenetic biomarkers are valuable and novel biomarkers that would be worth investigating before the appearance of occupational disease focusing on toxic agents and carcinogenic exposure. In the occupational field these biomarkers might: 1) add further information on the health status of the workers by integrating the novel biomarkers with the traditional and currently used biomarkers of effect, dose and susceptibility; 2) be applied as prognostic tool to prevent cancer risk in conjunction with periodical health survellance.

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