Unit 4 (a)

Inorganic carcinogenesis

Modes of Carcinogenesis

Cancer is an intricate disease that varies from person to person in development, appearance, and end point. Parallel to the disease complexity, cancer requires a multistep process for cells to undergo behavioral and metabolic changes, which will in turn prompt excessive and unnecessary growth and eventually lead to metastasis. The steps include mechanistic changes in the cells' ability to evade the immune defense, cell cycle pathways, and interaction with adjacent cells. There are two major modifications that can lead to cancer: genetic and epigenetic. Genetic changes such as mutations, translocations, copy number variations, sister chromatid exchanges, and karyotype variations are all important factors of cancer formation. Changes in the genetic sequence can accumulate and become permanent mutations, which can be neutral, harmful, or beneficial depending on the location and/or context. In fact, mutations are indispensable for the diversity among organisms. However, if these permanent DNA changes occur in essential cell cycle regulators such as tumor suppressor and promoter genes, the effects may be detrimental. Cell programming can also be disrupted through epigenetic changes, where structure rather than sequence of the DNA is altered. There are several different types of epigenetic mechanisms that can impact gene expression including DNA methylation, histone modification, and RNA-mediated silencing. The essence of the epigenetic principle lies in the fact that all cells share an identical genome; yet cells are able to demonstrate remarkably different functional and structural characteristics. Therefore, the role of epigenetic alteration on gene expression becomes ever so relevant. To date, the most common and well-understood epigenetic mechanism is DNA methylation, the addition of a methyl group to the 5-carbon position of the cytosine ring and in turn forming 5-methylcytosine. Cancer is generally exhibited as gene-specific hypermethylation and global hypomethylation. Hypermethylation is defined by heightened methylation in the gene promoter region, which serves to silence the expression. Hypermethylation (silencing) of tumor suppressor genes and hypomethylation (activation) of proto-oncogenes can both contribute to carcinogenesis. Posttranslational modifications of histones are regulated by histone acetyltransferases (HAT) and deacetyltransferases (HDAC), which add and take away acetyl groups, respectively. The importance of histone modifications and the correlation to cancer have been reported by multiple studies . One other form of epigenetic change is microRNA (miRNA). These small noncoding RNAs are thought to regulate up to 30% of protein-coding genes through targeted mRNA degradation and translational repression. Unsurprisingly, miRNA plays an essential role in cell proliferation and apoptosis . The deregulation of miRNA has also been shown in several types of cancers. Understanding the role of genetic and epigenetic changes in cancer has provided far-reaching knowledge not only for determining the mechanism of carcinogenesis but also the discovery of potential therapeutic targets.

Arsenic

Arsenic (As) is a chemical element classified as a metalloid. The most common oxidation states in the environment are +3 (As^{III}, also known as arsenite) and +5 (As^{V} or arsenate), which exhibit different grades of toxicity . Arsenic compounds can be found in organic (when linked with carbon and hydrogen) and inorganic (when combined with oxygen, chlorine, and sulfur, among other elements) forms.

Arsenic has been classified as a class I human carcinogen by the International Agency of Research on Cancer (IARC), meaning that there is sufficient evidence of carcinogenicity to humans.

Carcinogenic Mechanisms of Arsenic Exposure

Arsenic metabolism implicates a series of reduction and oxidation reactions. Pentavalent arsenical species are reduced to trivalent species, and oxidative methylation occurs to yield methylated tri- and pentavalent metabolites . However, more than a detoxification mechanism, it has been proposed that methylation can activate the toxic and carcinogenic potential of arsenic, since it has been demonstrated that mono/dimethylated arsenical species (both tri/pentavalent) can affect gene transcription, and are more potent enzyme inhibitors and cytotoxins than non-methylated species . Moreover, since the arsenic biotransformation pathway uses S-adenosylmethionine (SAM) as a methyl group donor, arsenic can also interfere with a number of cellular processes that require methyl groups, leading to the idea that alteration of epigenetic mechanisms can also participate in arsenic-induced carcinogenesis.

After reduction of As^{V} to As^{III} by purine nucleoside phosphorylase, $As^{III}$is methylated via a As^{III}-methyltransferase, using SAM as a methyl group donor [79], producing mono and dimethylated trivalent species, such as monomethylarsonous acid (MMA^{III}), dimethylarsinous acid (DMA^{III}), and equivalent pentavalent species (monomethylarsonic acid or MMA^{V}, and dimethylarsinic acid or DMA^{V}.

Arsenic-Induced Oxidative Stress

Cellular induced damage derived from arsenic biotransformation leading to carcinogenic processes have usually been described to occur through oxidative stress by generation of toxic species, such as reactive oxygen species (ROS) leading to genomic aberrations. Generation of ROS has been described as one of the earliest and most important mechanisms of arsenic-induced carcinogenicity . Oxidative damage (measured as guanine oxidation) is significantly associated with skin tumors associated with arsenic exposure. It has also been shown that oxidative stress can modify gene transcription profiles of human hyperkeratosis, affecting several cancer-relevant pathways, such as the Wnt/β-

catenin and calcium signaling pathways. Both single- and double DNA strand breaks are characteristic of most cancer types and have been shown to be induced by chronic arsenic exposure, even at low concentrations.

Arsenic, arsenic metabolites, and metabolism, directly and indirectly, affect normal epigenetic transcriptional regulation at both the level of DNA methylation, histone maintenance, and miRNA expression ,biotransformation and reduction of arsenic leads to the formation of highly toxic methylated arsenic species which act as potent cytotoxics and enzyme inhibitors. Since this process utilizes SAM, the cell's own methyl group donor, arsenic is thought to interfere with the cell's ability to maintain normal epigenetic regulation via the disruption of normal DNA methylation patterns, histone modification, and expression of microRNAs (miRNAs), possibly by the depletion of cellular pools of methyl groups . Epigenetic modifications do not alter the DNA sequence itself but instead result in chemical modifications to DNA or histone tail residues. Cells and tissues exposed to arsenic display epigenetic aberrations that mimic early hallmarks of cancer, providing evidence for an epigenetic role in arsenic-mediated tumorigenesis. Epigenetic regulation of gene expression is a highly dynamic process that can be modulated by existing therapeutics which may potentially apply to arsenic-related malignancies.

DNA Methylation

In the human genome, DNA methylation occurs at the 5-carbon position of cytosine in CpG dinucleotide sequences, resulting in 5′-methylcytosine (5mC), often within short evolutionarily conserved regions enriched for CpG dinucleotides, called CpG islands . When located in promoter regions of genes, CpG islands are typically unmethylated (~90%); however, promoters without CpG islands are frequently methylated . Therefore, the bulk of methylated DNA in the human genome occurs in repetitive DNA sequences, where it is thought to have an important role in silencing transposable elements and maintaining genomic stability. The transfer of the donor methyl group from SAM to the cytosine in a CpG dinucleotides is catalyzed by DNA methyl transferase (DNMT) enzymes, responsible for the de novo methylation throughout development (DNMT3a, DNMT3b, and DNMT3L) and maintenance of methylation patterns in somatic tissue (DNMT1). Aberrant DNA methylation is implicated in a vast spectrum of diseases and disorders and is one of the earliest and most frequent aberrations in cancer. DNA hypomethylation is associated with genomic instability and the reexpression of parasitic DNA, in addition to activation of genes normally silenced by methylation in a tissue-specific manner. Conversely, aberrant DNA promoter hypermethylation is strongly linked to transcriptional gene silencing, particularly for tumor suppressor genes (TSGs) and cancer.

Histone Modification

Histones proteins enable condensation of double-stranded supercoiled eukaryotic DNA into nucleosomes, which are made up of two copies each of H2A, H2B, H3, and H4

proteins. The N-terminal tails of histones are accessible to modifying enzymes, which function in catalyzing posttranslational modifications to the amino acid residues residing within the histone tail, including acetylation, methylation, ubiquitination, sumoylation, and phosphorylation amongst others.

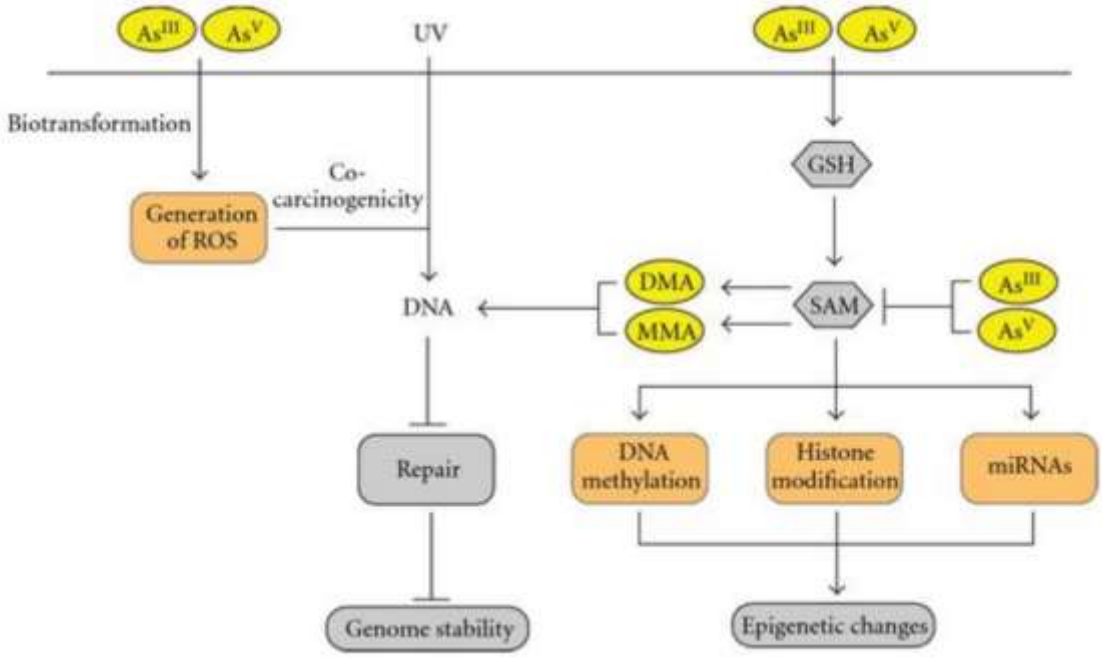
Arsenic compounds were also shown to induce malignant transformation of human nontumorigenic cell lines through changes to histone H3 acetylation, DNA promoter methylation, and decreases expression of the DBC1, FAM83A, ZSCAN12, and C1QTNF6 genes .

MicroRNAs

miRNAs are small, noncoding RNA species that orchestrate the expression of genes involved in many key aspects of cell biology by degradation and translational inhibition of their target mRNAs

An increasing number of studies show that arsenic exposure can alter miRNA expression levels in vitro and in vivo.

Overall, reviewed literature indicates that arsenic exposure exerts deleterious health effects primarily through the induction of oxidative stress, alterations to DNA methylation, histone modification, and miRNA expression.

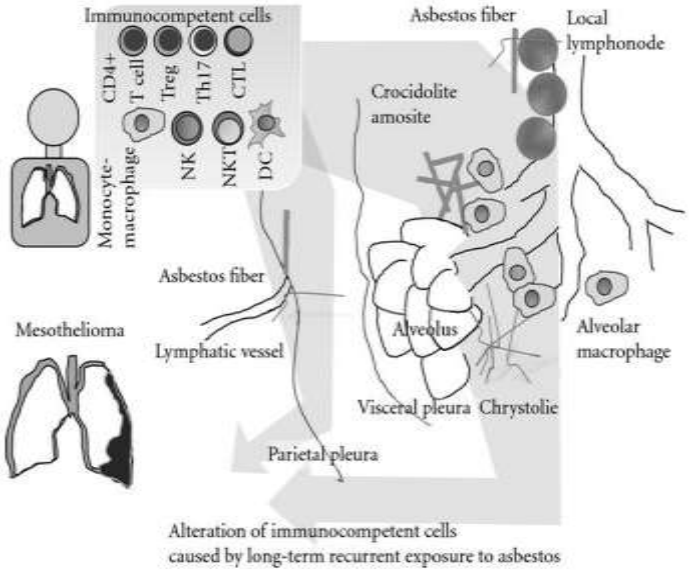


Asbestos carcinogenicity

Asbestos fibers have been heavily used in industry since World War II to the present because of their durability, heat-resistance, and low cost.1,2) However, in 1987, the IARC designated asbestos fibers as a Group I (definite) carcinogen for humans , and asbestos fibers were banned in many Western countries in the 1990.

Asbestos fibers Asbestos is a naturally occurring mineral conventionally divided into two mineralogic groups. The amphiboles include crocidolite (blue asbestos), amosite (brown asbestos), tremolite, anthophyllite, and actinolite. Among the amphiboles, only crocidolite and amosite have widespread commercial utilization. The noncommercial amphiboles (the most commonly occurring and widely distributed amphibole asbestos mineral group) are primarily significant as contaminants of other minerals, such as chrysotile.

Inhaled asbestos is usually handled by alveolar macrophages. The following cellular and molecular events then occur. (1) Capture of silica/asbestos by macrophages and entrapment within lysosomes. (2) Activation of NLRP3 inflammasome to cleave procaspase 1 to an active form. (3) Cleavage of prointerleukin (IL)-1β to an active form for release to form fibrotic nodules. (4) Production of reactive oxygen species (ROS) and reactive nitrogen species (RNS) in the macrophages. (5) Induction of cellular and tissue damage due to the production of ROS and RNS. (6) Apoptosis of the alveolar macrophages. (7) Production of various cytokines/chemokines such as IL-1βtumor necrosis factor (TNF)-α, macrophage inflammatory protein (MIP)-1/2, monocyte-chemoattractant protein-1, and IL-8 to cause chronic inflammation and proliferation of collagenic fibers. (8) Release of silica particles and asbestos fibers from alveolar macrophages and the repetition of similar cellular reactions described above by newly recognized nearby macrophages. (9) Transfer of silica particles and (partially cleaved) asbestos fibers to regional lymph nodes. (10) As these cellular and molecular reactions are continuously repeated, pulmonary fibrosis will appear gradually and progressively .



Mechanisms of carcinogenesis

The strong association between asbestos exposure and malignant mesothelioma has been widely accepted since 1960. Although asbestos is the primary etiologic agent for this tumor, a certain number of patients who develop mesothelioma have no known asbestos exposure. Radiation,) nonasbestos mineral fibers, organic chemicals, chronic inflammation,) and simian virus 40 (SV40) exposure have also been suggested as risk factors for mesothelioma in humans. Although the “SV40 oncogenic virus contamination theory” (polio vaccination) does not appear to be a major factor, at least in Japan,45,46) there is evidence that this oncogenic polyomavirus can play a variety of promotional roles in the carcinogenic processes of mesothelioma.) Currently, there are three hypotheses regarding the pathogenesis of asbestos-induced DMM, which may be summarized as follows: (1) the “oxidative stress theory”) is based on the fact that asbestos fibers are foreign bodies, and that epidemiological studies show asbestos fibers containing iron (a transitional metal which catalyzes free radical generation) to be more carcinogenic;) (2) the “chromosome tangling theory” postulates that asbestos fibers damage chromosomes when cells divide;) and (3) the “theory of adsorption of many specific proteins as well as carcinogenic molecules” states that asbestos in vivo contains chemicals including components of cigarette smoke.

In the “oxidative stress theory,” there are two distinct players, namely, endogenous iron in asbestos fibers and phagocytes. Regarding the former, surface iron can catalyze the production of reactive oxygen species.

Finally, as for the “adsorption theory,” it is well known that the surface of asbestos fibers have a high affinity for certain proteins and molecules. This appears to be at least partly associated with positive or negative charges on the asbestos surface.

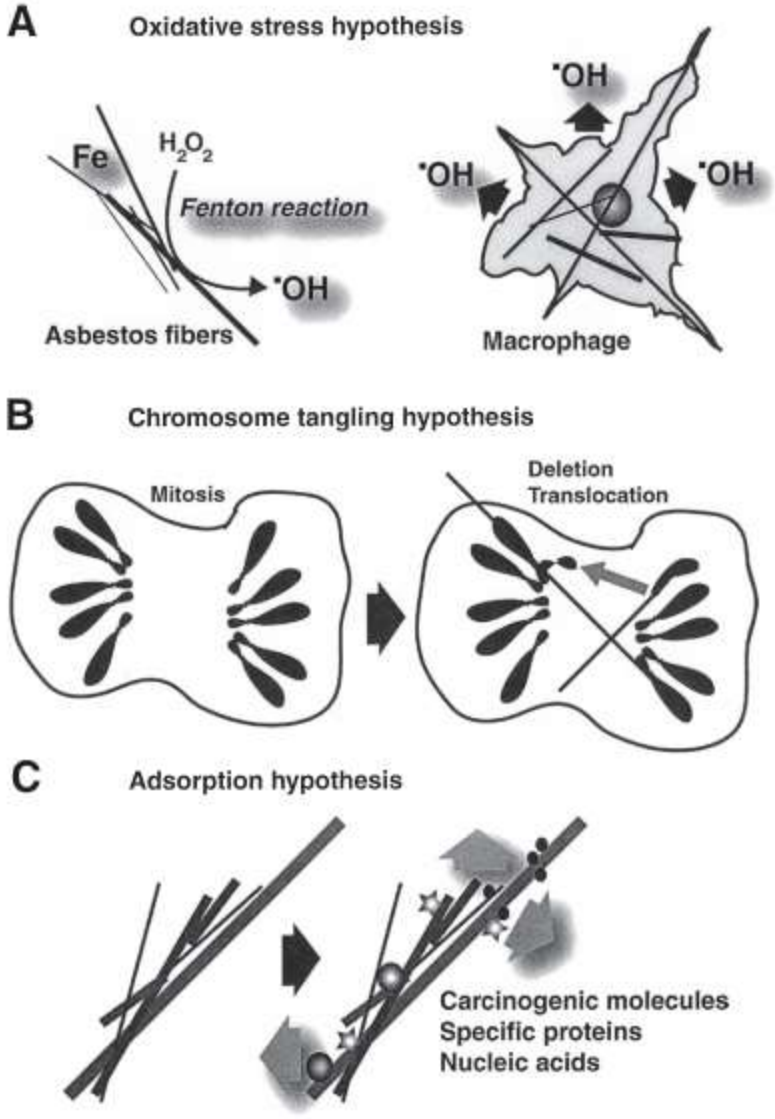


Fig. 3 Possible mechanisms of asbestos-induced carcinogenesis.

CHROMIUM (Cr)

Cr is abundant in the earth's crust, and its toxicity depends on its chemical state.60 It exists in divalent to hexavalent compounds, but only the trivalent and hexavalent compounds have significant biological toxicity.61–63 Cr compounds are usually found in industrial purposes such as chromite ore mining, pigment production, tanning of leather, formation of wood preservatives, and anticorrosive agents in cooking goods. Paint is a significant source of hexavalent Cr but is still used for industrial applications.

Toxicity and carcinogenic mechanism

The carcinogenicity of Cr dust has been studied since the 1980s. In a case study, lung cancer occurred more often in workers in the chromate-producing industry.

Trivalent compounds included in Cr dust are water-insoluble, but can enter cells in ionized form via a specific membrane transport system. High concentrations of trivalent Cr can lead to cellular damage. Hexavalent Cr is also a strong toxicant as it produces reactive hydroxyl radicals. In blood vessels, for example, Cr compounds are reduced from hexavalent to trivalent and reactive hydroxyl radicals are produced during the process. Thus, high levels of hexavalent Cr in the bloodstream cause blood cell damage by oxidation and functional degradation of the liver and kidney. If hexavalent Cr compounds are reduced to the pentavalent form, they can bind DNA and interrupt cellular processes. Furthermore, Cr in soil and water involve skin damage by absorption.

In order to understand the carcinogenic mechanisms of Cr, we investigated molecular pathway analysis using Pathway Studio showed that Cr and Cr compounds were mainly induce apoptosis, oxidative stress and DNA damage. We found Cr-related genomic interactions among nuclear factor (erythroid-derived 2)-like 2 (NFE2L2, Nrf2), TP53, BAX etc. Association between MT, which was mentioned with Cd toxicity, and Cr was also investigated. In addition, we also discovered that considerable diseases including lung cancer, skin allergy with dermatitis, and kidney diseases were induced by Cr and Cr compounds. These results may aid to comprehensive understanding of Cr-related toxicity mechanisms.

NICKEL (Ni)

Ni is widely used for industrial purposes because of its physicochemical properties. It is utilized in alloys and various products including rechargeable batteries, coins, electroplates, pigments, and stainless steel.

Toxicity and carcinogenic mechanism

Skin contact with Ni compounds through contaminated water, air, and children's toys result in dermatitis and allergy. Oral exposure to Ni also induces skin and oral epithelium damage.Industrial dust from Ni refineries contains water-insoluble Ni compounds including Ni_3S_2 and NiO, which are carcinogenic. Breathing in Ni-contaminated dust from Ni smelting, mining and tobacco smoking leads to significant damage to lungs and nasal cavities, resulting in occupational diseases such as lung cancer and nasal cancer in Ni refinery workers. Although the molecular carcinogenic mechanisms of Ni toxicity are not clear, several studies suggest that Ni

exposure induces oxidative stress via a reduction in expression of antioxidant enzymes and DNA single- and double-strand breaking.

Nickel is a hard, silver-white, malleable, ductile metallic element used extensively in alloys and for plating because of its oxidation resistance. Nickel, combined with other elements, occurs naturally in the earth's crust. Nickel released to the atmosphere typically exists in particulate form or adsorbed to particulate matter. Primary removal mechanisms of atmospheric nickel include gravitational settling and precipitation. Nickel released to soil may be adsorbed to soil surfaces depending on the soil conditions. Nickel released to aquatic systems generally exists in particulate forms that settle out in areas of active sedimentation. However, nickel also may exist in soluble form under appropriate conditions. Nickel salts exhibit significant solubility in water. Nickel occurs naturally in drinking water at an average concentration of about 2 μg/L. Adult daily intake of nickel from water is about 2 μg/day. About 170 μg of nickel is consumed in food per day. Available information indicates that nickel does not pose a toxicity problem following ingestion because the absorption from food or water is low. The most prevalent effect of nickel exposure is nickel dermatitis in nickel-sensitive individuals. Nickel dermatitis typically exhibits two components: (1) a simple dermatitis localized in the contact area and (2) chronic eczema or neurodermatitis without apparent connection to such contact. Nickel sensitivity, once acquired, may be persistent. Toxicological information of concern to industrially exposed humans is primarily confined to two potential categories of effects: (1) dermatoses, contact and atopic dermatitis, and allergic sensitization; and (2) cancers of the lung and nasal sinuses. Cancers of the lung and nasal sinuses in nickel workers have been described for more than 50 years in association with nickel refining processes (calcination, smelting, roasting, and electrolysis) and from nickel plating and polishing operations (e.g., electrolysis and grinding). Noncarcinogenic respiratory effects, such as bronchitis and emphysema, have also been seen in occupational exposures. These effects occurred at concentrations much higher than those found in the environment. .

In order to understand the carcinogenic mechanisms of Ni, we analyzed molecular pathway using Pathway, Ni induce apoptosis, oxidative stress, DNA methylation, and DNA damage. We investigated Ni-related genomic interactions among TP53, TNF, BCL2, etc. It is also discovered that various toxicity in lung, nose, skin, kidney and liver were induced by Ni. Interaction of MT and Ni was also investigated. This result may aid to comprehensive understanding of Ni-related toxicity mechanisms.

Genetic Mechanisms Underlying Nickel Carcinogenesis

Due to weak mutagenic potential found in mammalian cells, and even weaker response in prokaryotic assays, nickel compounds have generally been considered to be weakly mutagenic. The lack of mutagenic activity of nickel compounds in prokaryotic assays may be due to bacteria's inability to induce phagocytosis, a factor important for nickel toxicity. Another potential explanation for the stronger mutagenic potential found in mammalian cells is the number of DNA-associated proteins. Nickel's binding affinity for proteins is substantially higher than for DNA; thus the genotoxic effects would be greater in mammalian cells due to heightened interaction . In these mammalian studies, nickel compounds have been found to induce both mutations and chromosomal aberrations.

Nickel compounds have been found to have slightly positive mutagenic effects in a number of forward mutation assays using various cell lines such as V79 and mouse lymphoma cells. Deletion mutations were detected from these studies and have been deemed as a potential mutagenic mechanism for nickel. Occurrence of mutagenesis on an autosomal gene is justifiable because allelic chromosome can compensate for the loss from large deletions. The finding that nickel sulfide induced strong mutagenic response in an autosomal gene, transfected bacteria gpt gene, supports this notion . In addition to nickel's ability to promote mutations, other potential genetic mechanisms of nickel carcinogenesis include chromosomal aberrations, DNA-protein cross-links, and DNA base damage .

Epigenetic Mechanisms

Despite some evidence of nickel's weak mutagenicity, the recent advances in understanding the mechanism of nickel carcinogenesis have shifted toward epigenetic alterations. Epigenetics is defined as the inheritable and reversible changes in gene expression without changing the DNA sequence. DNA resides in a highly compact structure called the chromatin. The accessibility of the chromatin, depending on its closed or open structure, is essential for biological functions such as replication, translation, gene expression, etc. Epigenetic alterations such as DNA methylation, histone modification, and small noncoding RNA are critical factors in inducing changes in the chromatin structure. DNA methylation is correlated with repetitive sequence suppression, X-chromosome inactivation in imprinting, and long-term transcriptional silencing. Although methylation in the promoter region is linked to suppression due to interference with the binding of transcription factors, gene body methylation usually signifies activation. In In mammalian cells, approximately 60–90% of 5′-cytosine-phospate- guanine-3′ (CpGs) are methylated. DNA methyltransferases (DNMTs) are responsible for transferring a methyl group from S-adenosyl methionine to the fifth carbon of cytosine. CpG sites are regions of the DNA where cytosine is followed by guanine. CpG islands contain methylated CpGs about every 15 nucleotides as opposed to the rest of the genome where CpGs occur every 80–100 nucleotides. DNA methylation-induced gene inactivation is associated with numerous human diseases such as fragile X mental retardation and various types of cancers.

Nickel has also been found to inhibit dioxygenases, a family of enzymes essential for a balanced epigenetic landscape, and requires iron, oxygen, ascorbate, and alpha-ketoglutarates as cofactors Nickel has been shown to target the iron-binding motif of dioxygenases due to its effectively higher affinity than iron. The resulting irreversible inhibition of dioxygenases led to remarkable increases in DNA methylation marks. Other than DNA methylation, nickel can also trigger gene silencing through histone modifications. Histones are alkaline proteins in which the DNA winds around and forms nucleosomes, the basis of chromatin. There are five major families of histones: H1, H2A, H2B, H3, and H4. H1 serves as the linker histone connecting nucleosomes and forming higher order structures, while the other four are essential core histones. The N-terminal tails protruding from the nucleosome beads have more than 60 different residues, each of which can be altered by posttranslational modifications such as acetylation, methylation, sumoylation, phosphorylation, biotinylation, and ubiquitination [58, 82]. Of the above posttranslational modifications,

histone acetylation is one of the most extensively studied topics. Histone acetylation is a very dynamic phenomenon and is balanced by the contrasting activities of histone acetyltransferase (HAT) and histone deacetylase (HDAC). HAT serves to transfer an acetyl group from acetyl coenzyme A (AcCoA) to an e-amino group of a lysine residue. Upon the acetylation of a lysine residue, the positive charge of the histone side chain is removed, subsequently decreasing histone's affinity to the negatively charged DNA. The loosely bound DNA will then become more accessible to transcriptional factors in the promoter region.