Effects of Methyl Isocyanate on Patho-Physiology of Various Diseases – An Epigenetic Paradigm

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ABSTRACT

Epigenetic process are one of the most diverse & dynamic concept, which is controlled by a group various highly specialized cellular events like DNA methylation (5-methyl cytosine, 5-hydroxymethyl cytosine, 5-fluoro Cytosine, 5-carboxyl cytosine), histone modification (acetylation, methylation, phosphorylation, sumoylation, biotinylation, citrulination, ubiquitinylation, krotonilation, isomerization, GlcNAcylation, etc.), ATP-dependent chromatin remodeling, non coding RNA’s etc. They together are responsible for maintaining different epigenetic states which further contributes towards promotion of cellular diversity thereby regulating cell growth and its survival. These processes are involved in regulating gene expression states expressive, repressive & poised. Toxic industrial chemical exposure have been shown to influence the epigenetic mechanism in humans & epigenome acts as an interface between external environment & the genome therefore these epigenetic modification like DNA methylation, histone modification etc. are good harbingers for studying effects of external environment. Such misregulation could effectively contribute toward genome wide instability promoting various genetic abnormalities & cancers. Methyl isocyanate (MIC) is a noxious industrial chemical used extensively in the production of carbamate pesticides which was accidentally released during the Bhopal gas tragedy which resulted in death of thousands of individuals. Even after a lot of extensive studies on effects of MIC on the affected population, the clear mechanism of pathogenesis remains elusive. Studying epigenetic phenomenon might help unravel a new dimension & further enhance understanding related to patho-physiology of affected population.

Keywords: Epigenetic, Methylation, Carbamate, Genome, Cytosine.

INTRODUCTION

The term “epigenetics” is defined as a covalent modification of DNA, protein, or RNA, that results in alterations of their function and/or regulation, without altering their primary sequences. In simpler words, ‘epigenetics’ refers ‘above the genetics,’ indicate the changes in gene expression that occurs without any alteration in the DNA sequence. Epigenetic modifications are stable in several conditions and may be inherited on to future generations or transgenerational, however in other cases they are dynamic and alter accordingly to environmental cues. In 1950, Conrad Waddington has proposed the classical definition as “an epigenetic trait is a stably heritable phenotype resulting from alterations in a chromosome without changes in the DNA sequence”. Epigenetics act at the interface between the environment and genome. Several epidemiological studies conducted in humans reported the association of prenatal and early postnatal environmental factors in the development of various non communicable chronic diseases, such as cancer, cardiovascular disease, diabetes, obesity, behavioral disorders, schizophrenia etc. Increasing evidence from
animal studies also suggest that prenatal and early postnatal environmental factors including nutritional supplements, xenobiatic chemicals, behavioral cues, reproductive factors and low dose radiation may lead to altered epigenetic programming and resultant changes in the susceptibility of developing disease. As a matter of fact, prenatal and postnatal exposures could be linked to phenotypic changes later in lifetime where the alteration of epigenetic marks plays a key role in determining the functional information that is stored in the genome. Notably, epigenetic alterations are inherited mitotically in somatic cells, offering a potential mechanism by which environmental effects on the epigenome can have long term effects on gene expression. The outcome of these animal and human studies support the hypothesis of the fetal basis or developmental origins of adult onset disease. This theory validates the evolution of developmental plasticity, which enables an organism to acclimatize to environmental signals in early life, may also increase the susceptibility of developing chronic diseases. Developmental plasticity occurs as evident when environmental exposure creates a broad range of adult phenotypes from a single genotype by epigenetically altering gene expression.

Epigenetic process controls a number of cellular events by switching genes on or off, thereby modulating gene expression. It controls the fate of cells during development which gives rises to an array of specialized set of cells from a single cell. There are a number of important physiological processes which encompass various epigenetic modifications such as bookmarking, imprinting, X chromosome inactivation, position effect, maternal effects, paramutation, reprogramming, transvection, gene silencing and activation (LINE SINE, Alu elements, etc.), carcinogenesis, etc. Epigenetics is also associated with various disease states. Numerous epigenetic modifications have been discovered till date which controls the overall physiological activities of an organism. These include DNA methylation (5-methyl cytosine, 5-hydroxymethyl cytosine, 5-fluoro Cytosine, 5-carboxyl cytosine), histone modification (acetylation, methylation, phosphorylation, sumoylation, biotinylatation, citrulination, ubiquitinylation, krotonylation, isomerization, GlcNAcylation, etc.), ATP-dependent chromatin remodeling, non coding RNA’s.

Involvement of Epigenetics in Human Diseases

Epigenetic alteration includes an array of molecular modifications in DNA and chromatin. Among these, the most widely investigated mechanism is DNA methylation, which occurs at the 5-carbon position of cytosine in CpG dinucleotides, and alteration to the chromatin packaging of DNA by post translational histone modifications. Other epigenetic mechanisms also involve the control & regulation by non coding RNAs, and higher level organization of chromatin within the nucleus, which have a wide range of effects on gene expression. Epigenetic regulation also controls tissue specific gene expression & also maintains gene silencing of repetitive (transposable) elements, inhibiting their replication and transposition which can give rise to genome wide instability through insertional mutagenesis. The foremost studied epigenetically regulated process in mammals are X-chromosome inactivation and genomic imprinting.

Aberrations in DNA methylation is the key contributor to disease. For e.g. imprinting diseases viz. the Angelman, Silvere Russell, Pradere Willi and Beckwithe Wiedemann syndromes are normally related with perturbation in DNA methylation. Human diseases are attributable to DNA methylation based imprinting disorders. However, these genetic diseases including diabetes, schizophrenia, autism and cancers are associated with aberrations in imprinting. Abnormalities of the enzymes that cause DNA methylation also contribute to disease as reported by the rare Immunodeficiency centromere instability facial anomalies (ICF) syndrome initiated by mutations in DNA methyl transferase 3B (DNMT3B). Similarly, Rett syndrome is related to mutations in the methyl binding domain (MBD) protein and MeCP2 that leads to dysregulation in gene expression and neuro developmental disease. Aberrations in DNA methylation manifests itself in two forms either through DNA hypomethylation or hypermethylation. DNA hypomethylation mediates chromosomal instability and also leads to activation of oncogene, both of these features are extensively associated with oncogenesis, and DNA hypermethylation is often related to inactivation of tumor suppressor gene during tumor initiation & progressions.

Histone modifications frequently contribute to disease development and progressions. Histone
acetylation or deacetylation is one of the most common histone modifications that are involved in several diseases. Aberrations in histone modifications can seriously affect gene regulation, which is a common phenomenon in disease that could potentially be heritable across generations. Histone modifications are also associated with a number of diseases including cancer and neurological disorders. Crosstalk between DNA methylation and histone modifications can occur and both these factors may or may not affect epigenetic processes, which may lead to disease development.

Non-coding RNAs or micro RNAs (miRNAs), a promising area of epigenetics, and alternations in these RNAs potentially contribute towards a number of diseases. Although miRNAs have been related to various diseases such as Crohn’s disease, their exact role in tumorigenesis is now being studied and is considered to be a recurrent epigenetic aberration in cancer. Altogether, epigenetic mechanisms are now generally accepted to play a crucial role in human diseases.

In the mammalian genome, methylation occurs at cytosine bases located 5' to a guanosine in a CpG dinucleotide. This dinucleotide is characterized in the genome, especially short regions of 0.5–4 kb in length, known as CpG islands, are rich in cytosine & guanine content. Most CpG islands are present in the upstream promoter regions in majority (almost half) of the genes in the mammalian genome and are usually unmethylated in normal cells. In cancer, the hypermethylation of these promoter regions is a hallmark epigenetic change which are found in almost every type of human neoplasm and is associated with the inappropriate transcriptional silencing of genes. Promoter hypermethylation is involved in the disruption of classic tumor suppressor genes in human cancer. Around 50% of the genes cause hereditary cancers after undergoing mutation in the germ line are known to cause methylation associated silencing in various sporadic forms of cancer. Additionally, a panel of candidate tumor suppressor genes, O6-methylguanine-DNA methyltransferase (O6-MGMT) which encodes an important DNA repair gene, cyclindependent kinase inhibitor 2B (CDKN2B) which encodes p15 a cell cycle regulator and RASSF1A which codes for a protein of unknown function that can bind to the RAS oncogene are silenced by promoter hypermethylation in several cancers. These genes play a vital role in tumor initiation & progression on the basis of their presumed function, but seem not to be frequently mutated in such cancers. Promoter hypermethylation causes loss of function in many of these genes found in tumors. During the last decade, studies reported that aberrant promoter methylation is associated with a loss of gene function that can offer a selective advantage to neoplastic cells, as do mutations. For e.g. The von Hippel Lindau syndrome (VHL), breast cancer, early onset (BRCA1) and serine/threonine kinase (STK11) 11 genes germ line mutations of which cause hereditary forms of renal, breast and colon cancer, respectively
are frequently epigenetically silenced in the sporadic forms of these tumor types.37, 38

The significance of epigenetic silencing has etiology of non hereditary cancer is presented by studies of BRCA1. Earlier, this gene was considered as important for familial breast cancer (through BRCA1 germ line mutations). But, it is now clear that 10–15% of women with the non familial cancer have tumors in which this gene is hyper methylated. Besides, microarray studies suggest that overall gene expression profiles of sporadic breast cancers with hypermethylated BRCA1 are similar to those of the familial cancers in which BRCA1 is mutated and are distinct from those of other breast cancer types. The functional importance of the hypermethylation of tumor suppressor gene promoters allows checking the consequences of the inactivation of individual copies of such genes. Knudson’s two hit model predicts a phenotypic consequence of tumor suppressor gene which implies that the loss is not seen unless both alleles of a gene are inactivated in a tumor. A number of studies have shown that tumor can stably maintain mutations in one allele of a gene whereas the other allele is hypomethylated, leading to the functional inactivation of the gene. In fact, when one of the two alleles is mutated in the germ line of a patient with a familial cancer, and the resultant tumor holds both alleles of the gene, hypermethylation is usually seen as the second inactivating change. In addition to this, it is never present in the promoter of the mutated gene, but associated with the wild type allele. MLH1 (mutL homologue 1, colon cancer, non-polypsis type 2) is a mismatch repair gene frequently hyper methylated in sporadic tumors that have microsatellite instability. Significantly, these alterations in the methylation of the 5’ region of MLH1 was observed in the apparently normal colonic epithelium of patients that have colorectal cancer with microsatellite instability and also in hyperplastic regions, leading to the development of endometrial cancers that develop genetic change. MGMT is an additional DNA repair gene that is silenced and associated with promoter methylation in colon, lung, lymphoid and other tumors. O6-MGMT protein removes carcinogen induced O6-methylguanine adducts from DNA that results in G-A transition mutations, if not unrepaired. Tumors with silenced MGMT alleles also predisposed to mutation in key genes, such as tumor protein p53 (TP53) 23 and K-RAS5. This promoter hypermethylation, MLH1, precedes to genetic changes by occurring in premalignant polyps that do not harbor gene mutations. Another important finding with regard to tumor suppressor genes is that they are distorted epigenetically and/or genetically and are often found in genomic regions that are characterized by frequent chromosomal deletion, every chromosomal location is known to be a region that is normally deleted in human cancer. These deletions mediate loss of heterozygosity (LOH) and are commonly used to guide searches for tumor suppressor genes. Interestingly, there are LOH regions in which epigenetic events, rather than genetic alterations. For example, RASSF1A at 3p21 and hypermethylated in cancer 1 (HIC1), that encodes a transcription

<table>
<thead>
<tr>
<th>Disease</th>
<th>Epigenetic Aberration</th>
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<td>ATR-X Syndrome</td>
<td>Mutations in ATRX gene, hypomethylation of certain repeat and satellite sequences</td>
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<tr>
<td>Fragile X Syndrome</td>
<td>Expansion and methylation of CGG repeat in FMR1 5’ UTR, promoter methylation</td>
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<td>ICF Syndrome</td>
<td>DNMT3b mutations, DNA hypomethylation</td>
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<td>Rett Syndrome</td>
<td>MeCP2 mutations</td>
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<tr>
<td>Breast &amp; Ovarian Cancer</td>
<td>BRCA1, CpG island hypermethylation</td>
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<td>Melanoma</td>
<td>MAGE, CpG island hypomethylation</td>
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<tr>
<td>Colon Cancer</td>
<td>GATA 2 &amp; HOAX 2, CpG island shore hypermethylation</td>
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<td>Alzheimer's Disease</td>
<td>NEP, CpG island hypermethylation</td>
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<tr>
<td>Multiple Sclerosis</td>
<td>PADI2, CpG island hypomethylation</td>
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<tr>
<td>Rheumatoid Arthritis</td>
<td>DR3, CpG island hypermethylation</td>
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<tr>
<td>Systemic Lupus Erythematosus</td>
<td>PRF1, CD70, CD154, AIM2, CpG island hypomethylation</td>
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factor at 17 p13.3 which has two chromosomal regions characterized by frequent LOH in several tumor types are often hypermethylated in many important human cancers, viz. lung, prostate, colon and breast. These now constitute the major candidate tumor suppressor genes in these high frequency LOH regions, where no gene mutations are found consistently. The wide distribution of hypermethylated genes across the human genome, and the discovery of hypermethylated candidate tumor suppressor genes in regions of high frequency chromosome deletions, has urged the efforts to screen the cancer cell genome for such genes.

Epigenetics & Ageing

Epigenetic alteration drastically changes genomic stability, which in turn manifests themselves in several common age related diseases. The sirtuin family of protein deacylases, protects from several age related diseases and helps to maintain human body in a diseased free state. Sirtuins are involved in deacetylation of many substrates, including histones, and this activity is accelerated when the ratio between nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide dehydrogenase is high, thereby linking chromatin regulation to reduced food intake (dietary restriction) and exercise, two environmental interventions that delay aging. Recent evidence suggests that relocalization of sirtuins in response to DNA damage may drive epigenetic changes and genomic instability during aging. Sirtuins can also be activated by small molecule activators and by those molecules that raises the nicotinamide adenine dinucleotide levels. These substances mimic the effect of dietary restriction on genome wide gene regulation & expression.

Methyl Isocyanate (MIC): Toxic chemical intermediate

MIC is a highly inflammable clear, colorless liquid with a pungent odor that irritates the skin, eyes, and respiratory mucus membranes. It is made by reacting methylamine with phosgene. MIC is used in the production of pesticides, polyurethane foams and plastics and directly targets the lungs on inhalation. The odor threshold is approximately 100 to 250 times higher than the Occupational Safety and Health Administration (OSHA) limit of 0.02 ppm. Acute exposure to MIC vapors below the odor threshold can cause severe irritation to the eye and respiratory epithelium. Acute exposure to higher vapor concentrations may cause severe

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<th>Table 2: Reactions related to MIC molecules &amp; its physical / chemical properties (D'Silva et al., 1986)</th>
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<tr>
<td>In excess water: CH₃N=C=O + H₂O → [CH₂NHCOCOH] → CH₂NH₂ + CO₂</td>
</tr>
<tr>
<td>In excess MIC: CH₂NH₂ + CH₃N=C=O → CH₂NCONHCH₃</td>
</tr>
<tr>
<td>Molecular weight: 57.05 daltons</td>
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<tr>
<td>Boiling point (760 mm Hg): 102 °F (39.1 °C)</td>
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<tr>
<td>Freezing point: -49 °F (-45 °C)</td>
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<tr>
<td>Vapor pressure: 348 mm Hg at 68 °F (20 °C)</td>
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<tr>
<td>Vapor density: 1.42 (air = 1.00)</td>
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<tr>
<td>Water solubility: 6.7% at 68 °F (20 °C)</td>
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<tr>
<td>Flammability: highly flammable</td>
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<tr>
<td>Flammable Range: 5.3% to 26% (concentration in air)</td>
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pulmonary edema and injury to the alveolar walls of the lung even resulting in mortality. Survivors of acute exposures may show long term respiratory effects. Odors of MIC cannot act as harbinger of its toxicity because the (IDLH) limit deemed immediately dangerous to life or health is only 3 ppm while the threshold for detection of MIC vapors ranges from 2 to 5 ppm in humans. Most of the isocyanates are electrophilic in nature, they reacts with variety of nucleophilic reagents. Isocyanate and MIC reacts readily with the compounds bearing active hydrogen, these include hydroxylated compounds such as water, alcohols, phenols, and oximes, and also amino compounds. Water reacts exothermically with MIC to form 1,3-dimethylurea and 1,3,5-trimethylbiuret with evolution of carbon dioxide. Reaction of MIC in excess of water leads predominantly to the production of 1,3-dimethylurea & in excess of MIC in comparison to water produces 3,5-trimethylbiuret. MIC is also known to react with itself under a variety of conditions to form cyclic dimer and trimer, as well as linear polymers, with cyclotrimerization being the most common. It reacts with water to penetrate tissues, including skin & interacts with various amino acid residues. Humans exposed to MIC occupationally or accidentally may have an increased risk of developing respiratory disorders including asthma like allergy and inflammation, airway hyper responsiveness, reactive airway dysfunction syndrome (RADS) and eye infection. MIC is known for immunotoxicity, genotoxicity, reproductive and developmental toxicity. However, very little is known about the carcinogenicity of MIC in animals. On the other hand, accidental or occupational hazard of MIC in humans relating to carcinogenesis is still unknown. Besides, the information regarding susceptibility to carcinogenesis, cancer risk associated with MIC exposure also remains to be sparse. In contrast emerging in vitro evidences suggest genomic instability by MIC analogs in lung, liver and ovary epithelial cells that may undergo oncogenic transformation. Whether exposure to MIC induces a site specific cancer is still a controversy.

The Bhopal catastrophe

On 3rd December 1984 some 40 metric tons of MIC were accidentally released from Union Carbide India Limited (UCIL) plant, Bhopal, India. This catastrophic episode took a toll of 3600 human lives immediately and approximately 2,00,000 inhabitants were exposed to MIC. Amongst the survivors, 50,000 were expected to survive with long term effects. In 1985, Indian Council of Medical Research (ICMR) conducted a series of long term clinical studies, followed up for 10 years (till 1994) and finally declared that the effects of MIC are short term and that will not persevere in the exposed population. Since 1994, substantial debate suggested that both the exposed survivors and their offspring continue to suffer from the yet unknown long term effects.

Immediate Health Effects

Following the exothermic reaction of MIC, sudden physiological changes occur in the cellular and vascular components. The flooding of the respiratory tract and massive pulmonary edema are the cause for acute anoxic, rapid stimulation of the respiratory effects, cerebral edema and subarachnoid hemorrhage and eventually leading to mortality. The cherry red color, the changes in blood gases, such as lowered PO$_2$, lowered arterial and venous O$_2$ and CO$_2$ levels, the increase in DPG levels and increase of Hb levels and compensatory erythropoiesis are the effects of MIC. The available information about the high reactivity and binding of MIC end-terminal amino acids of blood and tissue proteins was virtually overlooked. However, the
uniqueness of MIC toxicity became apparent with the rapid development of chemical binding of MIC to end terminal amino and SH groups. Analysis conducted after Bhopal episode have found the presence of several toxic chemicals residues, including 1, 3-dimethylurea in E610 tank. MIC reacts exothermically with water to form carbon dioxide, methylamine, dimethylurea and/or trimethyl biuret.

Reproductive health effects

During the course of 20 years gynecological effects (menstrual abnormalities, vaginal discharge and premature menopause) was found to be of common prevalence among Bhopal MIC exposed women and their female offspring. Maternal fetal, gynecological effects have been reported through retrospective cohort studies. Girls who were exposed to MIC during their infancy and those in their mother's womb are suffering with 'menstrual chaos'. During an initial recovery phase, a comparative survey was conducted to find the effect of exposure to the toxic gas in pregnant women both in exposed and unexposed area in Bhopal. A higher occurrence of spontaneous miscarriages (24.2%) in the pregnant women exposed to the toxic gas was observed as compared to those in the control (unexposed) area (5.6%). No differences observed on other indices (adverse reproductive outcome, such as the rate of stillbirth and congenital malformations). The prenatal and neonatal mortalities were significantly higher in the affected area (6.9 and 6.1%, respectively), when compared to the control area (5.0 and 4.5%, respectively). The final technical report of ICMR has also found high miscarriage rates in the initial years after the disaster in addition to the increased menstrual irregularities and excessive bleeding among the gas exposed inhabitants. This pattern has been attributed to "post disaster trauma". Unfortunately, it is reported that several of these women had episodes of miscarriages later on, and many could not conceive at all. Another study found a relatively higher incidence of abnormal uterine bleeding and abnormal pap smears amongst the exposed women after 15 weeks of the exposure. An anthropometric study on exposed adolescents, conducted almost sixteen years after the disaster, found that there was selective growth retardation in boys, but not in girls, who had been exposed to MIC during their toddler age or those born to exposed parents.

Toxico-genomic effects

An initial study, reported sister chromatid exchanges (SCE) frequency in lymphocytes was increased more than three times in MIC exposed individuals. Chromosomal breaks were also observed in 10 out of 14 affected people (71.4%) studied, while only 6 out of 28 (21.4%) controls showed chromosomal breaks. Even chromatin bodies were observed in addition to the normal 46 chromosomes among some of the survivors. Another study reported chromosomal profile for 154 persons studied during 1986–1988. The MIC affected individuals developed at least two categories of chromosomal aberration, out of which Robertsonian translocation was repeatedly observed, mostly in acrocentric chromosomes 13 and 21. Such observations are suggestive of potential DNA damage by MIC. It is known that at least 50% of the subjects possessing such serious chromosomal abnormalities may have patho-phsiological implications such as tumors, recurrent miscarriage or transmission of defects to their offspring. A unique study conducted in 1990 clearly establishes genetic link of cancer patterns among gas victims of the tragedy with MIC exposure. Such studies were not conducted during the late recovery phase that would have helped identify people with chromosomal aberrations and at high risk of developing cancer.

Cancers

Several studies provide strong evidence regarding the association between MIC exposure and cancer. The morbidity data of human population exposed to MIC indicates gradually increased risk of cancers. Some of the cancers types reported in the affected population comprise of chronic lymphoid leukemia (CLL), Cancer of stomach, gall bladder, anal canal, glandular tissues, and sarcomas. The frequency of breast cancer was reported to be the highest among gas affected female population. The age group of distribution of breast cancer shows that the affected individuals were children at the time of exposure. Majority type of cancers reported in MIC affected population have a strong association with chemical exposure and reported earlier as evident in humans exposed to other chemicals and
carcinogens. Although, cancers of breast, lung, tongue and buckle mucosa were frequently reported in MIC affected population, further experimental and epidemiological studies are needed to authenticate on those cancers as the response to earlier MIC exposure induced effect.

**CONCLUSION**

Numerous studies conducted after the gas tragedy did not ponder upon the epigenetic aspect related to the health of affected individuals, although a number of interesting studies were conducted during these years but still the information regarding the epigenome of the MIC gas affected population & their posterity is still in its infancy. There are a number of reasons why analyzing epigenetic mechanism related to MIC related patho-physiology is of utter importance:

- The dynamic nature of epigenetic modification
- Epigenetic modification being operated at the interface between external environment & the genome
- Heritability of these modifications

There are also a number of studies conducted which have proved to be of immense importance in explaining the effects of environmental pollutants on the epigenetic mechanisms. Epigenetic modification are controlled by various proteins involved in DNA methylation / demethylation , histone modification, ATP depended chromatin remodeling, non coding RNA’S processes. Interactivity of MIC with with amino acids has already been reported by Cohen & Oppenhiemer. Epigenetic Proteome consist of hundreds of different & diverse proteins which together control the processes like gene expression, DNA repair, Cell cycle control, etc. therefore studying affects of various environmental pollutants such as MIC & its related products in particular the interactivity of various industrial chemical compounds with the epigenetic proteome might unravel a whole new understanding of effect of these chemical compounds on the epigenetic regulation.

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