Zinc Metabolism: A Review with Regard to Zn Finger Proteins, DNA Methylation and p53; In Reference to its Deficiency Syndromes and Tracing its Immunological as Well as Epigenetic Relations

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ABSTRACT: Epigenetics is the branch of biology that studies the effects of environment on genetics and vice versa. It is the connecting link between genotype and phenotype of an individual, and can be widely influenced by the nutrition and availability of certain essential micronutrients, in this review, zinc. Although a trace element, zinc is essential to the body as a core component of more than 300 proteins and enzymes, which when functioning normally impart structural and mechanical capacities to the body tissues and fluids. Zinc is ubiquitous to all parts of the body and regulates various metabolic and biological processes such as cell proliferation, apoptosis, tumorigenesis, along with the regular working of fetal and adult organs. The role of zinc-finger proteins is crucial to impart stability to a protein’s folds and in turn render it functional to play its role in gene expression to serve as oncogenes or tumor suppressor genes. Zinc deficiency is a common occurrence in various parts of the world and is generally due to inadequate intake through the diet but may also manifest itself in an inheritable form. The effects of low zinc on a cellular level can be seen through a diminishing immunity, increased oxidative stress, reduced functionality of the p53 protein leading to tumor formation, incorrect DNA methylation. While phenotypically, zinc deficit can be both congenital and acquired. Some such diseases discussed here are irritable bowel syndrome, acrodermatitis enteropathica, thymic atrophy, celiac disease and preterm birth. In this review, the focus is on the aspect of zinc availability to cells as an epigenetic modulator/regulator and the subsequent consequences arising due to imbalance of zinc in the cell.

KEYWORDS: Acrodermatitis enteropathica, Epigenetics, p53 protein, zinc-finger proteins.

1. INTRODUCTION TO EPIGENETICS

Comprehension of the epigenetic model require the famous clinical study of a pair of monozygotic twins with a congenital condition known as Kallmann’s syndrome. It is characterized by hypogonadotropic hypogonadism and anosmia. Mutation in the Kal I gene causes this condition in males where the olfactory placode of the embryonic brain is not fully developed [1]. As the name suggests, it impairs olfactory functions. Moreover, specific cells of this region play an essential role in the sexual development of the individual. Cells from the olfactory placode migrate to the hypothalamus during normal embryonic development. However, in individuals with Kallmann’s syndrome, this migration is impaired [2]. Thus, in theory, both monozygotic twins with this condition should have an impaired sense of smell and retarded reproductive development. However, in some peculiar cases, one twin is seen to have normal reproductive development while both remain olfactory impaired. The following graph shows the growth chart of the same [3].

Both individuals supposedly having the same genotype may manifest different phenotypes over the course of their lifetimes. This leaves a significant question on our understanding of how genes are transcribed and translated into proteins and the modifications they may undergo upon interacting with various other physiological and biochemical constraints and factors. Waddington first observed that the environment had the capacity to influence the genotype, something similar to the Lamarckian hypothesis of inheritance of acquired characters [4]. However, today we know that the same genotype is capable of manifesting different phenotypes upon interaction with the environment and, in some cases, may even be inheritable. Thus, a new branch of study emerged, studying the relationship between genes and the environment as well as the consequences of those interactions [5].

Apart from the adiabatic influences such as temperature, humidity, air pressure, and exposure to radiation. Food has a significant influence on the development and general biology of an organism. It is through this medium that the precursors of all metabolites are introduced inside the body, whereas the deficiency or excess of specific components in food manifests themselves as obvious
symptoms and pathologies. A lot of these changes, if not all, can very well be grouped as epigenetic in nature, and their study is revealing novel details about the biological systems [6].

2. MECHANISMS INVOLVED IN EPIGENETIC REGULATION
DNA methylation, histone modifications, and post-transcriptional changes in gene expression based on microRNA interference are all examples of epigenetic control.

Expression of gene
Gene-environment interactions are nonlinear and may include both direct and indirect activities. Numerous heritable dispositions, such as single nucleotide polymorphisms (SNP) or allelic translocations, are linked to a wide range of complicated disorders. As a result, they have occupied a central place in contemporary biomedical research, and interest in them has begun to grow. [7].

Methylation and histone modifications
The expression of a gene can be altered without changing its nucleotide sequence but by changing the folding of chromosomes, configuration of histones and/or packing of chromosomes [8] as shown in figure 1.

![Figure 1: Schematic representation of the function of 5,10-methenyltetrahydrofolate reductase (MTHFR) in the metabolism of folate, one-carbon transfer reactions, and biological methylation processes, including DNA methylation. The MTHFR C677T polymorphism, which results in decreased MTHFR activity and increased thermolability of MTHFR, leads to lower levels of 5-methylTHF and an accumulation of 5,10-methyleneTHF because MTHFR catalyses the irreversible conversion of 5,10-methenyltetrahydrofolate (5,10-methyleneTHF) to 5-methyltetrahydrofolate (5-methylTHF). Both MTHFR and cystathionine b-synthase are allosterically inhibited by SAM. B12, vitamin B-12; BHMT, betaine:homocysteine methyltransferase; CbS, cystathionine b-synthase; CH3, methyl group; CpG, cytosine-guanine dinucleotide sequence; DNMT, DNA methyltransferase; MS, methionine synthase; SAH, S-adenosylhomocysteine.](image-url)
3. INTRODUCTION TO EPIGENETIC FACTORS

3.1 Nutrition as a major epigenetic factor

The metabolic pathways for nutrients are encoded in DNA. Consequently, mutations might cause disruptions in the breakdown of a particular chemical, such as in galactose or fructose intolerance. Nonetheless, the control of gene expression is as crucial and is directly impacted by dietary constituents. The methylation of DNA, a significant regulatory mechanism, is a well-known example of the epigenetic effects of a food. Folic acid, vitamin B12, betaine, and choline are all methyl group suppliers. It was discovered that supplementing pregnant mice dams with these nutrients altered the coat color of their progeny [9].

3.2 Nutrition and immunity

Interleukin 8 and the IL23/IL17 pathway play a crucial role in the development of chronic inflammation, such as Crohn's disease (CD) and Inflammatory Bowel disease. Dietary techniques may have the potential to modulate inflammatory disorders [10].

3.3 Nutrition and ageing

It is recognized that genetic disposition, environmental influences, individual lifestyle, and dietary factors interact with the ageing process. The balance between hereditary and epigenetic influences on the development of cancer seems to shift with age. Whereas the majority of children malignancies are linked with an inherited genetic or epigenetic (e.g., imprinted) load, this ratio switches in favor of acquired epigenetic and genetic hits in adult and elderly tumors [11]. There are two types of influences on the ageing process: intrinsic and extrinsic. Intrinsic ageing (cellular ageing) is mostly determined by an individual's genetic background. External factors such as smoking, excessive alcohol intake, and poor diet contribute to extrinsic ageing. During ageing, epigenetic modifications occur, such as a reduction in global DNA methylation in contrast to an increase in CpG island hypermethylation. Important factors of cellular senescence and organism ageing are epigenetic alterations [12].

3.4 Epigenetic inheritance

Due to their intrinsic plasticity, epigenetic systems contribute to the modulation of gene expression in response to environmental inputs. Considerable evidence demonstrates that dietary imbalance and metabolic disruptions during important periods of development may have a lasting impact on health and may even be transferred to the next generation [13]. In conjunction with other parts of the contemporary synthetic theory of evolution, such as Evo-Devo, epigenetic inheritance may assist to better explain environmental impacts, evolutionary velocity, and discontinuity [14].
4. ZINC AN ESSENTIAL MICRONUTRIENT
Zinc is an element essential to the human body due to its structural and regulatory functions. Being a key micronutrient that is acquired from the diet, zinc is seen in almost all body tissues and fluids forming an integral part of multiple proteins and enzymes essential for a variety of activities viz. metal metabolism, cell cycle regulation, apoptosis, maintenance of normal fetal and adult functions [15]. It is generally used as a mineral supplement or multivitamin and as a therapeutic in maintenance of certain diseases like Wilson’s disease (due to its copper blocking effect) [16]. Zinc, when compared to its other metal counterparts, poses to be relatively non-toxic to humans. Acute zinc toxicity is a rare case, only when ingestion reaches 4-8g [17].

5. ZINC DEFICIENCY
According to the RDA, the recommended daily adult intake of zinc is 15mg. In the human body, skeletal muscles account for 60% of total body mass and weight, of which, 30% zinc is utilized here (FAO) [18]. Through this, it is evident that Zinc plays a vital role in the various biological and chemical processes occurring within the body. Zinc deficiency is a common occurrence in various parts of the world and may manifest in a variety of forms after a prolonged period of low zinc levels viz. changes in p53 activity linked to cell proliferation, epigenetic modifications, retarded puberty, inflammatory bowel disease, skin diseases (acrodermatitis enteropathica), thymic atrophy, celiac disease, preterm birth risk etc. All these conditions are linked directly to the presence of low amounts of zinc in the body that affects the metabolism of other related elements along with the formation of key enzymes important to regular functioning and maintenance [19].

6. ZINC, p53 and DNA
Studies show that a large population of cancer can be prevented if adequate attention is paid to nutrition, particularly zinc due to its importance in host defense and progression of cancer [20]. Being a structural component of chromatin, zinc has a significant impact on DNA and subsequently its replication, transcription and repair. There are more than 3000 transcription factors that are associated with zinc in the form of zinc-fingers (ZNF) and DNA-binding proteins. The p53 gene is a tumor suppressor gene on chromosome 17, which produces the p53 protein [21]. During cell proliferation, the p53 protein binds DNA. This binding stimulates the secretion of p21 protein that in turn reacts with cdk2 to halt the cell from passing into the next stage. Structurally, p53 has 393 amino acids and three functionally distinct domains, the most important being the DNA binding domain (DBD) that binds a single zinc ion [22]. Zinc deficiency in the diet directs an inadequate supply to the DBD, which in turn leads to tumorigenic mutations of p53, due to misfolding of the protein and destabilization by increasing its unfolding rate [23]. An increased unfolding rate causes aggregation in the cell along with decreased DNA-binding specificity. p53 is largely involved in the DNA repair machineries as well as in providing the DNA resistance against UV damage either directly or through p53-associated factors [24]. In a zinc deficient cell, p53 will fail to be functionally correct and neither the DNA repair process will be completed nor will p21 secretion be induced. This will thus lead to an uncontrolled proliferation of the cell along with the damaged or mutated DNA leading to the formation of a tumor, which further takes the shape of cancer [25].

7. EPIGENETIC MODIFICATIONS
DNA methylation is a heritable mark of epigenetic change in gene expression that is not encoded by the DNA. DNA methylation is essential for normal development as it plays a key role in a number of processes including genomic imprinting, X-chromosome inactivation etc [26]. It is well known that zinc plays a central role in zinc finger proteins (ZNF) that contain zinc atom(s) at their core binding site during DNA methylation and histone [27]. Zinc deficiency, thus, disrupts the enzymes’ functioning and results in epigenetic dysregulation.

7.1 Zinc in DNA methylation
The most prevalent epigenetic modification is the addition of a methyl group at the 5’ position of a cytosine base to maintain an overall genomic stability, preserve cellular identity and control gene expression. Insufficient zinc levels cause pathological consequences during incorrect methylation. One such is in the functioning of MTR (5-methyltetrahydrofolate-homocysteine methyltransferase). This enzyme is expressed in various tissues and organs and functions by facilitating the transfer of a methyl group from 5-methyltetrahydrofolate to homocysteine by employing zinc, cobalamin and MTRR enzyme (catalyst). The dysfunction
of this enzyme due to gene mutations or zinc deficiency may cause hyperhomocysteinemia that in turn may cause arterial damage and clotting of blood vessels [28].

7.2 Zinc in Zinc Finger Proteins (ZNF)
A small protein structural motif characterized by one or more zinc ions (Zn²⁺) is called a zinc finger protein that acts to stabilize the protein folds. They are involved in multiple cellular processes and have a key role in tumorigenesis, cancer progression and metastasis [29]. Zinc finger proteins are essential in various parts of the body where continuous cell proliferation occurs viz. skin, muscle, adipocytes, GI tract etc. Through their importance in gene expression, zinc finger proteins also serve as oncogenes or tumor suppressor genes and play a crucial role in cancer onset and its subsequent progression (ZNF281 that influences metastasis in colorectal cancer by regulation of epithelial - mesenchymal transition, ZNF750 which is involved in squamous cell carcinomas in liver, lung, cervix, ZNF185 in prostate cancer, ZBP89 or ZNF148 regulates cancer growth & apoptosis to name a few) [30]. Apart from its importance as an oncogene, ZNFs, through recent studies are now known to be crucial for the pathogenesis of certain neurodegenerative diseases (ZPR1 in spinal muscular atrophy, ZNF746 in Parkinson’s disease) as well as in the onset of puberty in primates through their hypothalamic expression [31]. A deficiency in zinc would cause the onset of or failure to regulate these few named diseases out of the several ones that are dependent on the proper functioning of zinc fingers to stabilize the protein folds that may then decide the fate of the tumor or disease of concern. In regards to this, the drugs that are being designed for the treatments of these diseases target specific ZNFs to restore the normal functioning or halt the abnormal expression of the core proteins expressing the disease [32].

8. ZINC IN THE INTESTINE
The small intestine has a major role in maintaining zinc homeostasis as the absorption of zinc occurs from this part of the body. Zinc forms complexes with proteins, fats and phosphates to maintain the normal concentration in the body and an imbalance may cause various types of abnormalities. Metallothionein (MT) and zinc transporters maintain the extracellular and intracellular cytosolic zinc levels [33]. Metallothionein is a protein metallochaperone (MC) that aids in the buffering of zinc ions into the cell. The MC protects against misfolding by sequestering zinc during the critical early stages of folding and zinc ions are transferred to the protein of concern only after a high-affinity pocket has been formed post correct folding [34]. MTs along with zinc work to protect against various inflammatory conditions. The deficiency of zinc and the reduction in the buffering capacity of MTs leads to a deficient uptake from the intestine, causing mutations and disorders viz. acrodermatitis enteropathica (AE), irritable bowel syndrome (IBS) and celiac disease (CD) [35].

8.1 Acrodermatitis enteropathica (AE)
Acrodermatitis enteropathica is a rare autosomal recessive genetic disorder that occurs due to the incapacity of the intestines to absorb zinc. It occurs due to an autosomal recessive mutation of SLC39A4 gene on chromosome 8 that determines a partial or total deficiency of zinc transporter protein zinc-ligand binding protein 4 (ZIP), in which the ability of the intestine to absorb zinc is impaired [36]. The effects of AE are only seen after the infant has weaned off mother’s milk, indicating that the presence of low molecular binding agents increased the bioavailability of zinc to the child [37]. AE manifests itself as skin lesions, severe diarrhea, alopecia, neuro-psychological disturbances and reduced immune function that led to the death of the individual in absence of treatment [38].

8.2 Irritable bowel syndrome (IBS)
IBS is a common disease, affecting ~12% of the population. It is a functional disorder without any detectable lesions in the patient’s [39], but is characterized by recurring abdominal cramps, bloating, constipation and/or diarrhea. Studies by Hujoel in 2019 concluded that the deficiency of zinc might perpetuate the underlying pathophysiology of IBS and play a role in the integrity of the gastrointestinal barrier. This is indicative that zinc supplementation may be a potential therapy to mitigate as well as improve symptoms of IBS [40].

8.3 Lethal milk in mice
Lethal milk (lm) is a recessive mutation on chromosome 2 occurring in inbred strain of mice where the milk produced by the dam is deficient of zinc and lethal to all the pups. The newborn pups suckling on this milk develop zinc deficiency and die within a week
before being weaned off [41]. However, if the pups are nursed to a normal dam or if the lm dam is given zinc supplements, the pups survive. The surviving pups show less symptoms of zinc deficiency in later stages of life.

9. PRETERM BIRTH

Preterm birth is a major contributor to prenatal mortality and may be as a result of genetic variation. During fetal development, zinc is required for many functions related to fetal growth. A maternal deficiency in zinc may lead to a variety of complications such as preeclampsia, preterm birth, reduced birth weight, congenital anomalies [42]. SLC30A (a zinc transporter (ZnT) in mammals) is spread all over the intestine to facilitate the absorption of zinc and regulate it. A mutation in this gene increases the risk of preterm birth due to low bioavailability of zinc to the fetus that affects the fetal splanchnopleure and causes low birth weight along with neurodegenerative disorders [43].

CONCLUSION

In the age of exponential development and growth the advent of the scientific age has debunked a multitude of age-old myths about human health and disease. However, any generalization or establishment of a rule requires thorough study and sufficient data. Initial discoveries in the field of genetics suggested that the code is pre-written inside every cell, while the ones prior to this discovery relied almost wholly on the environment for their explanation of a biological phenomenon. The latest findings suggest that it is a complex interplay of both the environment and the genes that determine the fate of an organism. The WHO report Genomics and World Health (WHO, Geneva, 2002) underlined that “Except for genetic diseases that result from a single defective gene, most common diseases result from environmental factors, together with variations in individual susceptibility, which reflect the action of several genes.” In context to the studies conducted upon zinc as an epigenetic factor, it can be easily derived that the presence of zinc as an essential micronutrient is very important and has a significant impact on functional proteins and enzymes. Not acquiring an adequate amount of zinc leads to disorders ranging from acute to chronic depending upon the physical condition of the individual under study and the level of deficiency of zinc. Better understanding of the interdependent biological pathways will help in targeting diseases with more precision, drugs can be tailor-made for specific groups of individuals. However, a lot of data needs to be generated and analyzed for that, the increasing computational capabilities show promising prospects in this regard. The high cost of collection of data remains the key-limiting factor in this field of research.

Conflict of interest: Authors declare no conflict of interest.

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