Heavy Metals and Epigenetic Alterations in Brain Tumors

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Abstract: Heavy metals and their derivatives can cause various diseases. Numerous studies have evaluated the possible link between exposure to heavy metals and various cancers. Recent data show a correlation between heavy metals and aberration of genetic and epigenetic patterns. From a literature search we noticed few experimental and epidemiological studies that evaluate a possible correlation between heavy metals and brain tumors. Gliomas arise due to genetic and epigenetic alterations of glial cells. Changes in gene expression result in the alteration of the cellular division process. Epigenetic alterations in brain tumors include the hypermethylation of CpG group, hypomethylation of specific genes, aberrant activation of genes, and changes in the position of various histones. Heavy metals are capable of generating reactive oxygen assumes that key functions in various pathological mechanisms. Alteration of homeostasis of metals could cause the overproduction of reactive oxygen species and induce DNA damage, lipid peroxidation, and alteration of proteins. In this study we summarize the possible correlation between heavy metals, epigenetic alterations and brain tumors. We report, moreover, the review of relevant literature.

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INTRODUCTION

Heavy metals are commonly present in the environment [1]. Prolonged exposure to these elements, including arsenic, nickel, lead, and cadmium, has been associated with various diseases, such as cancer, cardiovascular and neurological diseases [2]. A potential link between DNA methylation and heavy metals has been recently reported [3-5]. The production of reactive oxygen species (ROS) by metals induces the formation of free radicals [6]. Oxidative DNA damage alters the activity of methyltransferases resulting in an abnormal methylation of cytosine residues at CpG sites [7, 8].

Epigenetic is the study of heritable modifications in gene expression not due to changes in the primary DNA sequence [9, 10]. Epigenetic mechanisms can modify genome function under exogenous influence, permitting a continuous propagation of gene activity to the next cell generation. Indeed, modifications of DNA methylation processes and histone alterations can induce a progress in various diseases such as cancers, and neurological diseases [11]. In cerebral gliomas, genetic alterations cause the dysregulation of cellular cycle and progression of neoplastic lesion. However, the epigenetic mechanisms in gliomas remain poorly understood and are object of various experimental investigations.

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Recent literature has evidenced cancer incidence, including gliomas, in subjects with prolonged exposure to heavy metals such as lead, nickel, chromium, and cadmium [5, 6, 12]. Carcinogenic metals can alter various cellular processes causing DNA damages with oxidative and nonoxidative mechanisms. The present study summarizes the pertinent literature.

GENETICS OF BRAIN TUMORS

Gliomas are the most common primary brain tumors in adults. In the WHO classification [13] gliomas are divided into four different histotypes. Grade I gliomas show a slow proliferation rate. Grade II gliomas show an important cellular differentiation and could present a malignant progression. Grade III lesions evidence many mitotic cells and cellular atypia. Grade IV tumors include glioblastoma (GBM) and gliosarcoma and present microvascular proliferation and pseudopalisading necrosis [13].

The mechanism that leads to gradual neoplastic transformation through the transition from low-grade to high-grade gliomas is characterized by several mechanisms, including the mutation of the P53 gene. The effects of this genetic alteration are the loss of cellular regulation, the abnormal expression of cycline dependent kinase 4 and 6 (CDK4 and CDK6) and of ubiquitin ligase Mdm2 and Mdm4 [14].

The biological events that stimulate the gliomas' development are not totally understood. The gliomagenesis is characterized by numerous molecular processes involving
increased or altered production of many growth factor receptors [15-18]. Primary GBM shows amplification of the epidermal growth factor receptor (EGFR), deletion or mutation of homozygous cyclin-dependent kinase (CDK) inhibitor p16INK4A/CDKN2A, alterations in tumor-suppressor phosphatase and tensin homologue (PTEN) on chromosome 10, and deletion in the INK4a [16]. Secondary GBM is associated with deletion of chromosome 10, which includes PTEN. PTEN is a negative regulator of the phosphoinositide 3-kinase (PI3K) pathway, a major signaling pathway that stimulates cellular proliferation [19].

The P53 tumor suppressor gene controls the cell cycle by allowing the processes of DNA repair and removal of altered cells [20]. The effects of the mutation of the P53 are the loss of cellular cycle regulation, the abnormal expression of CDK4, CDK6, VEGF and FGFRβ [20]. The deletion of NFKBIA (encoding nuclear factor of κ-light polypeptide gene enhancer in B-cells inhibitor-α), promotes tumorigenesis in GBM that do not show alterations of EGFR [16]. Bredel et al. observed, in a large series of 790 human GBM, that NFKBIA was frequently deleted in GBM [21].

The PI3K/Akt pathway is a regulator of tumor cell metabolism, growth, proliferation, and survival [15]. The tumor-suppressor PTEN negatively regulates the PI3K pathway by dephosphorylating phosphatidylinositol-3,4,5-triphosphate (PIP3) back to phosphatidylinositol-4,5-bisphosphate (PIP2) [22].

The isocitrate dehydrogenase 1 (IDH1), is an enzyme present in the cytoplasm and peroxisomes, that induces the process of reduction of NADP+ to NADPH. Genomic analysis has demonstrated the mutation of amino acid 132 of IDH1, in over 70% of patients affected by gliomas [23].

Mutations of the ATRX gene have been found in gliomas and were shown to refine the prognosis of malignant gliomas in combination with IDH and 1p/19q status [24]. The ATRX gene is located on chromosome Xq21.1 and regulates the incorporation of the histone variant H3.3 at pericentric heterochromatin and at telomeres [25]. ATRX has been associated with altered patterns of DNA methylation, chromosomal alterations, and telomeric dysfunction [26]. Mutations of ATRX occur frequently in grade II astrocytomas (67%), grade III astrocytomas (73%), secondary GBMs (57%), and in mixed tumors of astrocytic and oligodendrocytic lineage (68%), whereas they are rare in primary GBMs (4%) [27].

**EPIDENETICS OF BRAIN TUMORS**

Epigenetic abnormalities frequently affect many biological mechanisms including cellular cycle regulation [29]. Because of their reversible nature, epigenetic alterations are under observation for the development of new therapeutic strategies. Features of cancer epigenetics are DNA methylation, nucleosome remodelling, and various processes of acetylation, methylation, and histones modifications.

The process of methylation is regulated by three principal DNA methyltransferases (DNMTs) [30, 31]. DNA methylation involves the covalent bond of a methyl group to the carbon-5 position of cytosine (C) to structure the 5-methylcytosine (5-mC), in cytosine-guanine (CpG) dinucleotides [32]. The process of cytosine methylation of CpG dinucleotides is often related to the inhibition of mechanisms of transcription [32]. Generally, neoplastic cells show, at the same time, complete hypomethylation and regional hypermethylation; instead, the process of hypermethylation occurs in specific gene-associated CpG areas unmethylated [30]. The processes of hypermethylation promote gene silencing regulators of various biological events such as DNA repair, apoptosis and angiogenesis. Primary GBMs are often linked to the demethylation and transcriptional activation of the oncogene MAGEA1 [33]. The MGMT gene regulates a DNA repair enzyme that removes alkyl adducts from the O6-position of guanine. Methylation of MGMT gene’s promoter makes cancer cells more responsive to the alkylating agent’s effects [34] and, at the same time, represents a predictive factor of favorable survival in GBM patients [34]. IDH1 mutation causes the inhibition of demethylation of DNA, and the accumulation of methylated DNA [35, 36].

Epigenetic alterations of histone affect the integrity of the genome and the generic expression. Histones are nuclear proteins that package DNA into nucleosomes [30]. The N-terminal tracts of histones are subject to many modifications, such as acetylation, methylation, phosphorylation, ADP-ribosylation [37]. In genomic analysis of GBM, various alterations of the histone were evidenced. Frequently, in response to alteration of the regulatory genes have been demonstrated an important aberration of the histone deacetylases 2 and 9 (HDAC2 and HDAC9) [37]. In GBMs than in low-grade astrocytomas, the expression of mRNA is decreased, while the histone 3 appeared more acetylated [38]. BMI-1 protein regulates histone H3K27 methylation; the epigenetic alteration of the gene that controls the encoding of the protein BMI-1 is associated to a poor prognosis [39]. Moreover, the epigenetic alteration of the encoding of BMI-1 also inhibits the Ink4a/Arf locus, stimulating cell proliferation [40]. A recent study has demonstrated that, in pediatric GBMs, the recurrent mutations in H3F3A affect amino acid substitutions at two positions within the histone tail (K27M, G34R/G34V) [41]. GBMs characterized by the H3F3A/G34 mutation are mainly localized in the cerebral hemispheres, and show high rates of mutation in P53, ATRX and DAXX [41]. On the other hand, GBMs that have the H3F3A/K27 mutation show a median localization, a high incidence of TP53 mutation, and DNA hypomethylation [41]. These cases are burdened with a poor prognosis [41].

MicroRNAs (miRNAs), are potential epigenetic regulatory effectors, and their dysregulation expression has been observed in various types of tumors. MiRNAs are segments of 19-25 nucleotides which can modify gene expression through interactions with mRNAs, blocking translation of mRNAs [42]. Reduced levels of miR-21 cause caspases activation and apoptosis [43]. In an experimental study, was observed, in human GBM samples and in GBM cellular lines (A172, U87, U373, LN229, LN428, and LN308), an overexpression of miR-21. The abnormal expression of mir-21 inhibited the expression of regulatory genes and apoptotic activity, both at the same time being responsible for the malignant progression of the tumor [43]. Aberrant increased expression of miR-21 promotes glioma invasive phenotype, by regulating genes that control in glioma cells, apoptosis pathway, migration and invasiveness processes, including the RECK and TIMP3 genes, inhibitors of matrix metalloprote-
inases [44]. In another study was demonstrated a decrease of cell proliferation as a result of reduced expression of miR-128 [45]. MiR-124 and miR-137 stimulate the cell cycle arrest in glioma cells; in fact, their expression in high grade gliomas is significantly reduced [46].

**EPIGENETIC MECHANISM OF HEAVY METALS**

Homeostasis of metal ions is obtained through complex mechanisms of uptake, storage and secretion [4, 5]. However, chronic exposure to metals can cause toxic effects and it is a concentration-dependent phenomenon [4, 5]. Toxic metal ions compete with indispensable ions for biological binding sites, altering the function of various molecules and the metal homeostasis [47]. The ability of metals to produce ROS and consequently to modify cellular redox states are believed valid mechanisms in metal-induced carcinogenicity [48, 49]. Intracellular accumulation of ROS and reactive nitrogen species induces a cellular redox disproportion that is often linked to carcinogenesis [50]. Oxidative stress causes the production of H$_2$O$_2$, precursor of the ‘OH radical, which, successively, can diffuse freely in the cells and in the tissues [51]. DNA mutations, instability of the genome, strand breakage, bases alterations and cell death are events linked to oxidative DNA impairment [52]. Oxidative stress damages bio-macromolecules, including proteins and lipids, induces various pathological conditions such as cancer, cardiovascular diseases, diabetes, Alzheimer’s and Parkinson’s disease [51, 52]. Moreover, recent studies show that the oxidative stress can also induce epigenetic alterations, and abnormal cellular growth [5, 8]. Generally speaking, DNA methylation, histone alterations and components of chromatin represent potential mediators of epigenetic inheritance.

Arsenic is largely diffused in the soil and in water. From the data highlighted by various studies it can be stated that prolonged exposure to this element is related to the processes of methylation of specific suppressor genes such as P15, P16, P53, and DAPK [53-55]. Recent data have shown, in subjects exposed to a prolonged contact with arsenite, hypermethylation of DNA in the promoter regions of the cyclin-dependent kinase inhibitor 2A (CDKN2A/p16INK4a), Ras association domain family protein 1A (RASSF1A) and serine protease 3 (PSS3) [56]. Trivalent arsenic has been correlated with reduced H3 and H4 lysine 16 acetylation in human bladder epithelial cells [57]. Nickel is a metal largely used in industry. Recent data demonstrate that the nickel processing causes a decrease in the expression of the DNA repair gene O6-methylguanine DNA methyltransferase (MGMT) in lung cancer cells [58]. Nickel also induces histone modifications including increases in H3K9 dimethylation, loss of histone acetylation in H2A, H2B, H3, and H4, and increases in H2A and H2B ubiquitination [59, 60]. More, nickel stimulate H3 phosphorylation, specifically in serine 10 (H3S10) via activation of the c-jun N-terminal kinase/stress-activated protein kinase pathway [61]. Cadmium (Cd) is used in metallurgy industry and in the production of batteries and pigments. Cadmium interferes with cell proliferation, differentiation and apoptosis pathways. Cadmium inhibits DNMT activities and induces in vitro DNA hypomethylation in TRL1215 rat liver cells [62]. Continued exposure to Cd causes the increase in activity of DNMT, hypermethylation of DNA, and the loss of expressiveness of tumor suppressor genes RASSF1A and p16 [63]. Moreover, some experimental studies evidenced the association between Cd exposure and miRNA expression [64, 65]. Scientific data show that exposure to cadmium legume Medicago truncatula causes the abnormal expression of six miRNAs, specifically the miR-393, miR-171, miR-319 and miR-529 were overexpressed while the miR-166 and miR-398 underexpressed [65]. Yet, through the analysis of new experimental data, in the genomic study of Brassica napus, was demonstrated the ability of cadmium to alter the expression of other micro RNA such as miR-156, miR-393, miR-171, and miR-396a [64]. Lead (Pb) is a high toxic metal that can be prepared in metallic and ionic form, and also as salt [66]. It residual long in water, soil, dust, and in manufactured products containing the metal. Food, air and drinking water are the major sources of lead exposure [66]. Lead is a genotoxic agent and induces DNA breaks and chromosome aberrations inducing oxidative stress. The formation of free radical can be directed including H$_2$O$_2$, and hydroperoxides or by reducing cell antioxidants [67]. In primates within the first year of life, who have taken constant amounts of lead, a marked decrease of the methyltransferases DNMT1 and DNMT3A was observed to be associated with important modifications of histones H3K9ac, H4K8ac, H4K12ac and H3K4me2 [68]. These epigenetic alterations would be responsible for abnormalities in the function of genes involved in brain activities such as neuron-derived orphan receptor 1 (NOR1), membrane associated phospholipase A2 precursor (PLA2) and flavoprotein subunit of complex II [68].

**HEAVY METALS AND BRAIN TUMORS**

Various studies highlight a correlation between industrial activities and cancer incidences. Heavy metals are soluble in water and can, therefore, be easily absorbed. In the living organisms, these metals bind to a large variety of bio-molecules damaging their functions. Recent evidences from epidemiological studies demonstrate that some neurological diseases, such as Alzheimer’s and Parkinson’s disease, may be correlated to heavy metals’ exposure. Although the exact mechanisms are still unclear, new data suggest that metals cause oxidative stress, neuroinflammation, and cellular death. The formation of radicals is increased in the brain because of the considerable oxygen metabolism of neurons [69-71].

Microglia are parenchymal cells capable of antigen presentation to T-cells that patrol the CNS. Microglia seem to facilitate glioma invasion digesting extracellular matrix components restricting tumor cell motility [72]. Tumor associated macrophage/microglia show a key role in the secretion of growth factors, cytokines and matrix metalloproteinases which represent the key angiogenic effector cells capable of modulating angiogenesis in gliomas [72]. Activated microglia release oxygen metabolites, reactive nitrogen species, and proteinases. Autopsy studies have shown an increase of CD14, expression of monocyte infiltration, in subjects chronically exposed to heavy metals [73]. Microglial activation by manganese chloride also induces neurotoxicity in vitro and the use of antioxidants, such as superoxide dismutase/catalase, glutathione, or inhibitors of NO biosynthesis effectively protected dopaminergic neurons [74]. Also, the blood-brain barrier that protects the brain parenchyma from
Numerous epidemiological studies have investigated the role of occupational exposures in the etiology of brain tumors but the results have been inconsistent. In a recent epidemiologic study has been evaluated the etiological role of occupation in gliomas development. The results showed an increased risk, particularly for low-grade gliomas, only for men engaged in the metal industry [76]. In 1970, a cohort of 413,877 Finnish women with blue-collar occupations was checked for the risk of cerebral tumors. Augmented risks were found for iron exposure (standardized incidence ratio, 2.15; 95% confidence interval, 0.96 to 4.80), chromium (1.51; 0.85 to 2.67), lead (1.27; 0.81 to 2.01), and cadmium (1.26; 0.72 to 2.22) [77]. A recent study confirms the carcinogenic effects of cadmium showing its effects on increasing the permeability of the BBB [78]. Various observations have demonstrated that prolonged contacts to arsenic or its compounds are correlated to major risk of lung, skin, liver, bladder, and brain cancers [79]. Lead could facilitate the processes of carcinogenesis, altering the process of DNA synthesis and repair, inhibiting the activity of tumor suppressor proteins. Recent evidence suggests that lead is able to cross the barrier causing an increase of its values in the brain parenchyma [80]. In an epidemiological study, Steenland and Boffetta evaluated 6 occupational cohort concerning brain cancer risk in patients with prolonged metal exposure. The incidence of brain tumors was more evident in individuals chronically exposed to metallic elements [81]. In another epidemiological study the risk of developing brain tumors as a result of continued exposure to lead were evaluated using standardized mortality ratio and proportional hazards and Poisson regression techniques, adjusting for the effects of age, gender and other covariates. The data obtained show an increased likelihood of incidence of brain tumors in those most exposed to lead [82]. Other research, in subjects exposed to lead, has documented alterations in the processes of DNA repair [83]. In addition, case-control studies of occupational exposures to lead report slight increased risk of cerebral tumors in the highest levels of lead [84, 85]. In other epidemiological studies, a significant association between meningioma risk and prolonged exposure to lead was found [86-88]. In a research study, the potential carcinogenic role of metals such as nickel, cadmium, chromium, arsenic, silicon and beryllium for human brain tumors was investigated. A statistically significant association was inferred between the development of cerebral tumors and the concentrations of silicon (p = 0.01), magnesium (p = 0.01), calcium (p = 0.03), and zinc (p = 0.05) [89]. In a recent study, the possible presence of heavy metal in patients affected by malignant glioma, was investigated. Analysis of serum concentrations of metals including Zn, Pb, Co, Cd, and Fe was performed by spectrophotometric examination. Serum Cd, Fe, Mg, Mn, Pb and Zn levels were increased in patients group compared to control group [90]. Mercury is an environmental toxicant that is correlated with brain toxicity. Humans can be exposed to methylmercury (MeHg), a neurotoxic organic form of mercury, by consuming contaminated seafood. MeHg shows a genotoxic activity causing damage to the central nervous system, and to the cardiovascular and renal systems [91]. However, a potential carcinogenicity activity of mercury in brain tumors is not well demonstrated. An epidemiologic study has revealed that occupational chromium exposure represents a risk factor for lung cancer, malignant lymphoma and brain tumor [92].

CONCLUSION
Malignant glioma treatment remains one of the most challenging areas of neurosurgery. Improvements in surgical technique, including intra-operative mapping, permit only a better management of gliomas. Radiation therapy and chemotherapy are only used as adjuvant therapy [18]. Targeted therapies are intriguing strategies able to upregulate the selectivity of therapeutic agents and restrict systemic toxicity. The information about the genetic bases of gliomas and their progression are poor. A large number of molecules, such as proteases, and cellular adhesion molecules show a key role in gliomas development [15]. Considering the large number of molecules and pathways regulating biology gliomas, the inhibition of a single target is not sufficient to conflict neoplastic progression [16]. Heterogeneity of the glioma cells, which includes expression of cellular surface receptors, as well as proliferative and angiogenic features, might be attributed to morphological and epigenetic plasticity. Understanding the mechanisms of these processes may help us in developing a better definition of gliomagenesis and of the biological events identifying novel molecular and most sensible targets. Risk factors for gliomas are largely unknown, except for hereditary syndromes such as neurofibromatosis, or tuberous sclerosis, as well as ionizing radiation to the head. Both inherited disorders and irradiation are rare occurrences, accounting for less than 10% of all gliomas and suggesting that complex genetic abnormalities combined with unknown environmental factors predispose individuals to glioma development [93]. Interactions and/or alteration of genes that interact with specific environment entities can occur at various genomic levels, including DNA, genes and chromosomes.

Prolonged exposure to heavy metals such as lead, nickel, arsenic, and cadmium is strongly correlated with an increased likelihood of malignancy and, specifically, for brain tumors as well as of disorders of the cardiovascular system and the renal system, and cognitive impairment in children. From experimental data, it is evident that exposure to carcinogenic metals can be correlated to alterations in the epigenetic profile. Experimental and epidemiological data have confirmed the potential carcinogenicity of certain heavy metals. This peculiarity is expressed through various epigenetic mechanisms such as alteration in the expression of specific genes, disorder in the processes of DNA methylation and inhibition of DNA repair processes. The production of free radicals and oxidative stress could represent the initiation of these processes. Objective of future research is represented by the discovery of new molecular pathways able to explain the correlation between heavy metals exposure and carcinogenesis.

CONFLICT OF INTEREST
The author(s) confirm that this article content has no conflict of interest.
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