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Aneuploidy as an Early Mechanistic Event in Metal Carcinogenesis

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Abstract

Aneuploidy has recently been proposed as an initiating event for carcinogenesis. There is significant evidence that carcinogenic metals induce aneuploidy. Here we review the mechanisms for how carcinogenic metals may induce aneuploidy and the evidence that carcinogenic metals induce an aneugenic effect which can destabilize the genome leading to genomic instability and cancer.

Introduction

Genomic instability, changes in chromosome structure and/or number, is a common feature of solid tumors. Specifically, aneuploidy occurs frequently in a variety of malignant tumors and is considered the most consistent marker of malignancy. It has been demonstrated in mild form in pre-neoplastic alterations and in more complex form in fully established neoplasms [1]. Aneuploidy has recently been proposed as an early or causative factor in several types of cancers such as cervical, throat, colon, lung, skin, pancreatic, gonad, esophageal as well as acute leukemia [2]. The role of aneuploidy as a causative event versus a consequence remains controversial. For example it does not explain carcinogenesis in diploid cancers [3]. Nonetheless, the aneuploidy theory more accurately meets the criteria for carcinogenesis than the traditional mutation theory [1,3,4] which, in particular, does not fit for the carcinogenesis of metals.

Random aneuploidy can be induced by a chemical agent or arise spontaneously [2,4]. The initial aneuploidy can then destabilize the numbers and structures of chromosomes, altering the balance of proteins that regulate chromosome segregation, DNA synthesis, and DNA repair which then leads to further instability [2–4]. Additionally, conditions of polysomy, monosomy or nullisomy in chromosomes which harbor tumor suppressor genes or

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oncogenes can significantly alter gene dosages and enhance tumor progression [3,4]. The more aneuploid the karyotype becomes the more unstable and aggressive the malignancy [3].

In order to investigate carcinogenesis through the induction of aneuploidy by chemical carcinogens such as metals, it is important to understand the potential mechanisms of how aneuploidy arises. There are several molecular mechanisms that can induce an aneuploid state. Several of these mechanisms have overlapping molecular targets and can thus induce aneuploidy by many potential pathways but three stand out as distinct mechanisms. First, supernumerary centrosomes can cause multipolarity during mitosis and subsequent missegregation of chromosomes. Centrosome abnormalities have been reported in most cancers including breast, bladder, brain, bone, liver, lung, colon, prostate, pancreas, ovary, testes, cervix, gallbladder, adrenal, and head and neck squamous cell [2]. The mechanisms for supernumerary centrosomes involve over-duplication, cytokinesis failure, centriole splitting and the formation of acentriolar centrosomes [5]. Second, the spindle assembly checkpoint (SAC) ensures that all kinetochores attach to mitotic spindles with proper tension. A significant number of proteins have been identified in the checkpoint which must all coordinate for proper chromosome segregation; this provides multiple targets for disruption by chemical carcinogens. Many of these proteins have been shown to be altered in cancer and have also been shown to be deregulated by chemical exposure [3,6–8]. Chemicals that induce microtubule defects can disrupt the SAC allowing progression to anaphase without proper attachment of all chromosomes. Third, failed cytokinesis or mitotic slippage can lead to tetraploid cells which can later destabilize to hyperdiploid cells [9, 10]. We will consider each of these mechanisms with respect to metal-induced carcinogenesis.

Carcinogenic Metals

Arsenic, beryllium, cadmium, chromium, cobalt, and nickel are widely considered as carcinogenic metals [11], additionally, lead has recently been determined to be a probable carcinogen [12]. The carcinogenic mechanism of action for most metals is largely unknown. Metals in general are considered to be weak mutagens, if mutagenic at all, and therefore traditional theories such as the gene mutation theory, which involves a series of mutations in tumor suppressor genes and oncogenes, do not fit for metal carcinogenesis. Here we review the evidence for aneuploidy and chromosome instability as the driving force for metal carcinogenesis.

Arsenic

Arsenic causes lung, skin, liver, and bladder cancer [13]. Several studies show it to be aneuploidogenic and to disrupt the normal progression of mitosis which can lead to polyploidy [14–20]. Several mechanisms have been proposed for the ability of arsenic to induce aneuploidy, but more study is needed to determine the specific mechanism as the data are conflicting and insufficient to draw a specific conclusion.

The first possible mechanism is effects on microtubules. The induction of lagging chromosomes [14,16] suggests a disruption of microtubule assembly dynamics; however, two more recent studies suggest that arsenic does not affect spindle formation [7,21]. The

second possibility is effects on the SAC, arsenic-induced mitotic cell death requires the activation of the SAC indicated by the activation and proper localization of BubR1, Mad2 and Cdc27 and the stabilization of securin and cyclin B, [7,17,18]. However, arsenic eventually bypasses the SAC, resulting in premature anaphase and the induction of diplochromosomes and tetraploidy in subsequent cell divisions [17]. Next, tetraploidy or the induction of 4N DNA content was evident in several additional studies [14,17,20,22], which can further destabilize to hyperdiploid cells causing imbalances of gene dosages. Finally, there is the centrosome amplification method. Centrosome amplification is induced by arsenic treatment, and it has been detected indirectly through the formation of multi polar spindles and directly by immunofluorescent staining of centrosomes [18,20,21,23,24,25]. Arsenite-induced centrosome amplification does not require dysfunctional p53, but the loss of p53 results in a significant increase in centrosome amplification which would lead inevitably to further instability [21,24]. Co-treatment with other carcinogens, such as nicotine-derived nitrosamine ketone (NNK) which reduces p53 in the nucleus, synergistically increases centrosome amplification [25]. However, more specific details in this mechanism are unknown.

Beryllium

Beryllium has long been associated with various lung diseases, including cancer [26]. However, very little research has been done on the mechanism of beryllium induced lung cancer [27]. Studies of the genotoxicity of beryllium have been inconsistent due to inter-laboratory differences in test systems and beryllium compounds [27]. No studies considered the induction of aneuploidy by beryllium. Consequently, currently nothing is known about the contribution of aneuploidy and its mechanism with regard to beryllium induced carcinogenesis however in vitro assays have shown that beryllium has the ability to suppress the dynamic instability of microtubules [28] suggesting a potential mechanism for an aneuploid effect.

Cadmium

Cadmium is a known human lung carcinogen and a suspected human kidney and prostate carcinogen [26]. A recent review of cadmium carcinogenesis outlines the roles of DNA repair inhibition, inhibition of apoptosis, induction of oxidative stress and aberrant gene expression [29]. It does not however address the contribution of aneuploidy though studies have shown that cadmium has a role as an aneugen. Cadmium has been shown to be a spindle poison [30] as well as an aneugen by both kinetochores positive micronuclei and chromosome counting [15,16,31]. The authors propose that this effect was due to malsegregation and not due to nondisjunction of chromatids [16]. Supporting this hypothesis, cadmium was shown to induce the SAC and lead to proteolysis of cdc20 and accumulation of cyclin A via activation of p38 signaling [8].

Chromium

Hexavalent chromium (Cr(VI)) is a well-established human lung carcinogen, but the mechanisms of chromium carcinogenicity are poorly understood [32]. Cr(VI) has been shown by multiple studies and multiple groups to be aneugenic as measured by both chromosome assays and centromere positive micronuclei assays [15,16,31,33–38]. The

aneugenic effect of Cr(VI) particles was significantly enhanced with continuing or prolonged treatments including an increase in polyploid or near polyploid cells [33–36]. The mechanisms for the aneuploidy are uncertain.

One study showed an increase of lagging chromosomes indicative of microtubule disruption [16], though this outcome has not been reported by others. Disruption of the SAC by Cr(VI) particles was manifested by the formation of chromosome spreads showing centromere spreading, premature centromere division, and premature anaphase [34,35]. In addition, levels of Mad2 expression were decreased indicating that the SAC was satisfied and the cells were allowed to proceed to anaphase [35]. An additional study considered the effect of cobalt-chromium alloy particles which also showed SAC bypass in the form of sister chromatid separation [38].

Centrosome amplification is a third potential mechanism for Cr(VI)-induced aneuploidy [33,34,39]. Zinc chromate, in particular, was shown to induce a prolonged G2 arrest along with an increase in centriolar defects [34]; this suggests multiple mechanisms for chromium-induced centrosome amplification such as multiple rounds of centrosome duplication during the prolonged G2 arrest, centriole splitting and acentriolar centrosome formation. Supporting a role for centrosome amplification in Cr(VI)-induced numerical chromosome instability, Xie et al 2007 [39] showed that cells neoplastically transformed by lead chromate exhibited increased levels of aneuploidy and centrosome amplification.

Cobalt

Cobalt is considered a probable lung carcinogen [40]. Little attention has been given to the contribution of aneuploidy with regard to cobalt induced carcinogenesis. A review article in 2003 reveals that cobalt chloride induces aneuploidy in lymphocytes, particularly in the D and G group chromosomes; hamsters intraperitoneally injected with cobalt chloride exhibited aneuploidy in bone marrow and testes; and cobalt refinery workers and hard metal workers showed increased chromosome loss [41]. Suggested mechanisms of carcinogenicity include DNA damage, DNA repair inhibition and spindle interference [41]. A more recent study showed that human skin cells treated with physiologically relevant doses of cobalt caused increases in both simple and complex aneuploidy and that the aneuploidy resolved over time in culture [37]. Interestingly, when cells were co-treated with hexavalent chromium the incidence of aneuploidy dramatically increased and the time to resolution was longer [37]. In addition, cells treated with a cobalt chromium alloy showed increases in chromosome loss, chromosome gain, polyploidy and sister chromatid separation [38].

Lead

Lead is considered to be a probable carcinogen and may act as a co-carcinogen [12]. Lead has been demonstrated to be pro-aneugenic in both cell cultures as well as epidemiologic studies [42,43]. In mammalian cell cultures non-cytotoxic concentrations of inorganic lead compounds induced a higher incidence of CREST positive micronuclei than CREST negative micronuclei demonstrating a tendency towards aneuploidy [42,44]. Based on cell free studies, this aneuploidy is likely a result of destabilized microtubules causing the assembly/disassembly steady state to shift to microtubule disassembly due to inhibition of

tubulin assembly [42,44]. In addition, lead was shown to affect microtubule motility which is likely to result in lagging chromosomes [42,44]. These results are consistent with a study showing that children with elevated blood lead levels had a higher rate of centromere positive micronuclei than centromere negative micronuclei as compared to a non-exposed group of children [43].

Nickel

Nickel is a known carcinogenic metal causing lung and nasal cancers largely in occupational settings [32]. Epidemiologic, animal, and cell culture studies have all implicated nickel compounds in carcinogenesis but the mechanism of action remains unclear. Several studies have addressed the ability of nickel to induce chromosome damage however; few studies have addressed the ability of nickel to induce aneuploidy. Studies in human cells showed an increase of kinetochores positive micronuclei after 24–26 h treatment of nickel sulfate and nickel chloride [31]. An additional study [45], showed that nickel did not induce aneugenic effects in hamster cells immediately following a 24 h exposure to nickel sulfate, however upon monitoring up to 72 h post exposure there were increases in the production of aneuploid metaphases, CREST positive micronuclei, as well as an increase in cells exhibiting errors in chromosome segregation including lagging chromosomes, chromatin bridges and asymmetrical segregation. This suggests that nickel may have effects on the spindle apparatus. This idea is supported by a recent genome wide screen of deletion mutants in *S. cerevisiae* investigating pathways affected by nickel sulfate [46]. Functional categories were identified which rendered cells either sensitive or resistant to nickel. Components involved in chromosome segregation and division were identified as a category in nickel-resistant strains; more specifically, proteins involved in the outer kinetochore were identified which are likely to lead to attachment defects suggested by the phenotypes seen in the cell culture study.

Other metals

Additionally there are other metals whose carcinogenicity is less understood that have evidence of aneugenic effects. Exposure to mercury can occur in a methylated form or as an inorganic metal. One study showed that methyl-mercury but not inorganic mercury can perturb the integrity of the microtubule organizing centers (MTOCs) leading to abnormal centrosome formation; induce multipolar cell division; and multi-nucleated cells; however no disruption of microtubules was detected [47]. In contrast, other groups using the same V79 Chinese hamster cell line, showed that inorganic mercury could cause inhibition of microtubule assembly and inhibited kinesin motility in vitro, as well as induction of aneuploidy as measured by CREST-positive micronuclei [44,48]. Another study showed depleted uranium induces centromere positive micronuclei, while enriched uranium induced more centromere negative micronuclei [49]. Vanadium interferes with chromosome distribution by inhibition of dynein ATPase and microtubule polymerization; in addition several studies reported induction of numerical chromosome aberrations and increased micronuclei [50].

Conclusion

All of the carcinogenic metals except beryllium have demonstrated an induction of aneuploidy in some fashion; aneuploidy has not yet been studied with beryllium. All of the metals do show a potential to disrupt microtubules which in turn can affect the proper segregation of chromosomes. The mechanisms of aneuploidy and their role in carcinogenesis has been fairly recently elucidated and thus the appropriate studies have not been done for all of the carcinogenic metals in order to say which mechanism predominates if at all. More basic research needs to be done on many of the metals. In sum, all of the carcinogenic metals show evidence of the ability to induce aneuploidy as an initial response to metal exposure thus beginning the cascade of aneuploidization and chromosome instability.

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