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Mechanisms of Metal-Induced Centrosome Amplification

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Abstract

Exposure to toxic and carcinogenic metals is widespread; however, their mechanisms of action remain largely unknown. One potential mechanism for metal-induced carcinogenicity and toxicity is centrosome amplification. Here, we review the mechanisms for metal-induced centrosome amplification, including arsenic, chromium, mercury and nano-titanium dioxide.

Keywords

Metals; DNA damage; centrosome amplification; arsenic; chromium; mercury

Introduction

Centrosomes are the microtubule organizing centers of the cell. They play an important role in cell division and the maintenance of genomic stability [1]. A normal mitotic cell contains two centrosomes oriented on opposite poles of the cell. These centrosomes are responsible for generating bipolar mitotic spindles which promote proper segregation of chromosomes into two equal daughter cells [1]. The daughter cells each contain one centrosome. This centrosome is duplicated during the next cell division cycle and its duplication is tightly coordinated with the DNA replication cycle. The strict coordination of the centrosome and DNA cycles ensure that the cell enters into mitosis with only two centrosomes [2]. However, cellular and genotoxic stress induced by metals can disrupt this linkage and promote centrosome amplification [3]. Cells that enter into mitosis with amplified centrosomes can produce multipolar spindles resulting in unequal chromosome segregation and aneuploidy [1]. Aneuploid cells can either survive and persist in cell population or undergo apoptosis in the next cell division and be removed from the population. Aneuploid cells that survive can ultimately lead to tumor formation and cancer [1].

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Mechanisms of Centrosome Amplification

Four major potential mechanisms for centrosome amplification have been proposed [as reviewed in 1]: 1) Multiple centrosome duplication cycles in one cell cycle, 2) failure of cytokinesis, 3) centriole splitting and 4) acentriolar centrosome formation. The first mechanism, multiple duplication cycles, occurs when the cell division and centrosome duplication cycles become uncoupled and the centrosome duplication cycle continues while the cell cycle is stalled [2]. This mechanism appears to be the most common and most studied mechanism for centrosome amplification in response to DNA damage. The second mechanism, failure of cytokinesis, occurs when the cell fails to undergo cytokinesis at the end of mitosis and enters into the next cell division cycle with two nuclei and two centrosomes [2]. Therefore, if the cell undergoes another cell division cycle it will enter into mitosis with four centrosomes instead of two. The third mechanism, centriole splitting, occurs when the two G2 centrosomes split to form four centrosomes with one centriole [2]. Lastly, acentriolar centrosome formation is the generation of centrosomes that do not contain centrioles [2]. The mechanism for acentriolar centrosome formation remains largely unknown.

Metal-induced centrosome amplification

Arsenic

The vast majority of research into metal-induced centrosome amplification has considered arsenic compounds. Arsenic is a known human lung and bladder carcinogen [4], but its carcinogenic mechanism remains unknown. Studies utilizing numerous cell lines show that multiple arsenic compounds induce centrosome amplification, including inorganic arsenic, dimethylarsinic acid and thio-dimethylarsinate [5–10]. Arsenic-induced centrosome amplification occurs in mitotic cells but does not appear to occur in interphase cells [7] and is associated with a prolonged mitotic arrest [5–7]. Arsenic-induced mitotic arrest and subsequent centrosome amplification and multipolar spindle formation requires the spindle assembly checkpoint as data show that inhibition of BubR1 and Mad2 attenuate arsenic-induced centrosome amplification [7,8]. In addition, data show that the amplified centrosomes caused by arsenic contain either abnormal centriole numbers or no centrioles at all, suggesting centrosome fragmentation and/or overexpression of pericentriolar proteins are involved in the underlying mechanism [7]. Arsenic-induced centrosome abnormalities appear similar to those seen after heat shock [8]. Inhibition of heat shock protein 70 and 90 (HSP70 and HSP90) induces a synergistic increase in centrosome amplification and mitotic arrest after arsenite exposure [8]. This suggests that HSP70 and 90 protect the cells against stresses induced by arsenic exposure.

Lack of a functional p53 protein appears to increase arsenite-induced centrosome amplification but p53 loss is not required for the induction of supernumerary centrosomes after arsenite exposure [8,9]. Liao et al. 2007 showed that cells with functional p53 exhibit a small increase in centrosome amplification after arsenite exposure but arsenite induces a significant dose-dependent increase in centrosome amplification in p53-inhibited cells. This increased centrosome amplification correlates with a decreased expression of p21 and Gadd45a in the p53 compromised cells [9]. Therefore, this study suggests that a

compromised p53 status along with reduced Gadd45a and p21 levels increases arsenite-induced centrosome amplification and neoplastic transformation. Taylor et al., 2008 also found that cells with compromised p53 exhibit higher levels of centrosome amplification, but cells with a functional p53 can still induce high levels of centrosome amplification [8] and, therefore, the loss of p53 is not required for arsenite-induced centrosome amplification.

Interestingly, the reports on centrosome amplification-induced cell death after arsenic exposure are contradictory. Yih et al., 2006 showed that the mitotic arrest and centrosome amplification observed after arsenite exposure led to mitotic death in cells with a non-functional p53 and reducing centrosome amplification could inhibit this mitotic cell death [7]. In contrast, Liao et al., 2007 demonstrated that cells lacking p53 exhibited reduced cytotoxicity and apoptosis along with increased soft agar colony formation as compared to cells with a functional p53 [9]. Recently these findings were extended to show that arsenic and cigarette smoke, specifically 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) exposure, can act synergistically to increase centrosome amplification and soft agar colony formation [10]. NNK exposure reduced arsenic-induced apoptosis by reducing the presence of p53 in the nucleus, thereby allowing cells with arsenite-induced centrosome amplification to survive [10]. The explanation for the differences in apoptotic response between these studies may lie in the cell lines used. Yih et al., 2006 used a HeLa variant cell line which is highly tumorigenic and exhibits an abnormal karyotype while Liao et al., 2007 used BEAS-2B cells, a non-tumorigenic cell line with a stable karyotype. Therefore, the explanation for this different apoptotic response may be due to alterations in additional pathways in the HeLa cell line.

Taken all together, the data indicate that arsenite likely induces centrosome amplification during a prolonged mitotic arrest induced by activation of the spindle assembly checkpoint, which is not reliant on p53 inactivation. However, tumorigenic cells lacking p53 undergo mitotic cell death in response to arsenite-induced centrosome amplification; while non-tumorigenic cells lacking p53 exhibit enhanced centrosome amplification, survival and soft agar growth after arsenic exposure.

Hexavalent Chromium

Like arsenic, hexavalent chromium (Cr(VI)) is a well-known human lung carcinogen and its carcinogenic mechanism is unknown [4]. Cr(VI) is often grouped into two major categories, water soluble and water insoluble (i.e. particulate). The available data strongly implicate the particulate form of chromate as the most potent carcinogenic form [11]. It has been suggested that one potential reason for the difference in potency between particulate and soluble Cr(VI) compounds may be due to the persistence of particles at lung bifurcation sites resulting in more prolonged exposures [12–14]. Studies using lead chromate as a model particulate Cr(VI) compound show that more prolonged exposures induce a concentration- and a time-dependent increase in aneuploidy and centrosome amplification in both interphase and mitotic cells in normal human lung fibroblasts that was not observed after only a 24 h exposure [12]. Lead chromate also disrupts normal mitotic progression inducing increases in cells exhibiting disorganized anaphase and mitotic catastrophe, phenotypes that both correlate with centrosome amplification [12]. Cells neoplastically transformed by lead

chromate also exhibit increased centrosome amplification and aneuploidy suggesting that centrosome amplification and subsequent aneuploidy may be involved in lead chromate-induced neoplastic transformation and ultimately carcinogenesis [15].

Recently these studies were expanded to investigate the effects of zinc chromate on centrosomes. Zinc chromate is another particulate Cr(VI) compound and epidemiology studies implicate it specifically as the most potent human lung carcinogen [16,17]. The data show that, similar to lead chromate, a 24 h exposure to zinc chromate does not induce aneuploidy or centrosome amplification, but more prolonged exposures do induce concentration- and time-dependent increases in aneuploidy and centrosome amplification in mitotic and interphase cells [13]. These data suggest that centrosome amplification and induction of aneuploidy is a characteristic of particulate Cr(VI) compounds in general.

Zinc chromate also induces numerous types of centriolar defects along with prolonged G2 arrest [13]. These data indicate that multiple mechanisms might occur to produce amplified centrosomes, including multiple rounds of centrosome duplication during a prolonged G2 arrest, centriole splitting and acentriolar centrosome formation.

The mechanism for how Cr(VI) amplifies the centrosomes during G2 arrest remains unknown. Previous studies show that particulate chromate induces DNA double strand breaks that are specifically generated in the G2 phase of the cell cycle [18–22]. In response to particulate Cr(VI)-induced DNA double strand breaks, ataxia telangiectasia mutated (ATM) protein, ataxia telangiectasia-related (ATR), Chk2 and MRE11 are activated [18–20,23] and are likely responsible for the G2 arrest observed after particulate Cr(VI) exposure. Studies performed using gamma-irradiation show that ATM and Chk1/2 is required for DNA damage-induced centrosome amplification, but they also suggest that a specific signal may be required to promote centrosome amplification rather than just a delay at G2 [24–26]. Two proteins that have been implicated in playing a role are CDK2, Rad51 and BRCA1, but the mechanism and other potential proteins remain unknown [24,27–29]. One mechanism that has been proposed is that activation of Chk1 in response to DNA damage leads to phosphorylation and subsequent activation of Cdk2 resulting in centrosome amplification [29].

The mechanism for particulate Cr(VI)-induced centriole splitting also remains unknown. It is likely that Cr(VI)-induced DNA damage may also play role in centriole splitting. Cells that enter into mitosis with DNA damage or incompletely replicated DNA can undergo centriole splitting generating multiple centrosomes with one centriole, multipolar spindles and abnormal cell division [30]. In addition, cells exposed to ionizing radiation exhibit a dose-dependent increase in centriole splitting [28]. Even though DNA damage is probably involved in centriole splitting, the mechanism still remains unknown.

Chronic exposure to particulate Cr(VI) also induces acentriolar centrosome formation. The mechanism for acentriolar centrosome formation, in general, is still not clearly understood. It has been suggested that acentriolar centrosomes may form in the presence of excess centrosomal components [2]. In addition, proper chromosome cohesion has also been implicated in maintaining spindle pole integrity [31]. Loss of proteins involved in

chromosome cohesion, including haspin, Sgo1 and Scc1, result in the generation of cells with a mixture of centriolar and acentriolar centrosomes [31]. Interestingly, the mitotic phenotype of cells depleted of haspin appear similar to the mitotic phenotype of cells that exhibit Cr(VI)-induced acentriolar centrosomes. More work is required to determine the mechanism of Cr(VI)-induced acentriolar centrosome formation, but chromosome cohesion defects may be a promising mechanism.

Mercury

Mercury is a dangerous and widespread environmental contaminant that can adversely affect multiple organ systems, particularly the nervous and renal systems [32]. In addition, the Environmental Protection Agency lists mercury as a possible human carcinogen [32]. Only one study has investigated the ability of mercury to induce centrosome amplification and reported that methylmercury, but not inorganic mercury, induced both a mitotic arrest and centrosome amplification in mitotic cells [33].

Nano-Metal Particles

Nano-metal particles are being used in a wide variety of products and applications, but their toxicity remains largely unknown. One study has investigated the effects of nano-metal particles on centrosome amplification using nano-titanium dioxide [34]. Long term exposure of human fibroblasts to nano-titanium dioxide for 12 weeks increased bi- and multinucleated cells, multipolar spindle formation and chromosome mis-segregation [34]. There was also a concurrent deregulation of mitotic Plk1 localization which may have altered mitotic exit resulting in multinucleated cells and the subsequent generation of centrosome amplification [34]. Thus, one potential mechanism for nano-titanium dioxide-induced centrosome amplification is failure of cytokinesis, but more study is needed.

Conclusion

Metal-induced centrosome amplification and its role in carcinogenicity and cellular toxicity is understudied. Currently, only four metals have been shown to induce centrosome amplification, arsenic, hexavalent chromium, methylmercury and nano-titanium particles and each metal appears to induce centrosome amplification via a different mechanism. Arsenic and methylmercury induce centrosome amplification during a prolonged mitotic arrest while nano-titanium particles appear to generate multiple centrosomes through failure of cytokinesis. Cr(VI) induces centrosome amplification via multiple mechanisms, but most likely as a result of DNA damage. Together, these data show that there is no uniform mechanism for metal-induced centrosome amplification. Therefore, it is important to continue to assess the mechanism of metal-induced centrosome amplification on an individual basis.

Abbreviations

HSP70	Heat shock protein 70
HSP90	heat shock protein 90

NNK	4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone
Cr(VI)	hexavalent chromium
ATM	ataxia telangiectasia mutated
ATR	ataxia telangiectasia-related

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