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Cadmium-induced malignant transformation of rat liver cells: Potential key role and regulatory mechanism of altered apolipoprotein E expression in enhanced invasiveness

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ABSTRACT

Cadmium is a transition metal that is classified as human carcinogen by the International Agency for Research on Cancer (IARC) with multiple target sites. Many studies using various model systems provide evidence of cadmium-induced malignancy formation *in vivo* or malignant cell transformation *in vitro*. Nonetheless, further studies are needed to completely understand the mechanisms of cadmium carcinogenicity. Our prior studies have utilized a rat liver epithelial cell line (TRL 1215) as a model for cadmium-induced malignant transformation. In the present study, we focused on the molecular mechanisms of this malignant transformation, especially with regard to hyper-invasiveness stimulated by cadmium transformation. By performing a series of biochemical analyses on cadmium transformed cells, it was determined that cadmium had significantly down-regulated the expression of apolipoprotein E (ApoE). ApoE was recently established as a suppressor of cell invasion. A key factor in the suppression of ApoE by cadmium appeared to be that the metal evoked a 5-aza-2′-deoxycytidine-sensitive hypermethylation of the regulatory region of ApoE, coupled with interference of the action of liver X receptor α (LXRα), a transcriptional regulator for ApoE. Furthermore, the expression of LXRα itself was suppressed by cadmium-mediated epigenetic modification. Re-expression of ApoE clearly abrogated the cell invasion stimulated by cadmium-induced malignant transformation. Together, the current results suggest that the cadmium-mediated enhanced cell invasion is linked to down-regulation of ApoE during malignant transformation these liver cells.

1. Introduction

Cadmium is a highly toxic transition metal, and is classified as a human carcinogen by the International Agency for Research on Cancer (IARC) (IARC, 2012). Many studies have shown the linkage between cadmium exposure and human cancer or cancer in rodent models, and *in vitro* cadmium has been associated with malignant cell transformation. There is evidence for relationship between cadmium exposure to human and cancer of the liver (Waalkes, 2000). Previous reports showed that cadmium induce tumors of the liver in rodents (Waalkes and Rehm, 1994; Waalkes, 2000). However, the precise mechanism or mechanisms of cadmium carcinogenesis are not well defined and further studies are required to completely understand its mechanisms. Since binding of cadmium to DNA is weak, cadmium is thought to be a heavy metal exhibiting an indirect genotoxic/mutagenic profile (Waalkes and Poirier, 1984; Waalkes, 2000). Epigenetic changes have been

Abbreviations: IARC, International Agency for Research on Cancer; ApoE, Apolipoprotein E; LXR, liver X receptor; LXRE, liver X receptor response element; 5-aza-dC, 5-aza-2′-deoxycytidine; ABCA1, ATP-binding cassette transporter; SREBP-1c, sterol regulatory element-binding protein-1c.

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shown to be involved in the cadmium-induced carcinogenesis; for example, both the degree of genomic DNA methylation and the enzyme activity responsible for DNA methylation (DNA methyltransferases) are increased by long-term cadmium exposure (Takiguchi et al., 2003; Benbrahim-Tallaa et al., 2007; Jiang et al., 2008). Moreover, expression of tumor suppressor genes or genes involved in apoptosis was known to be down-regulated through their DNA hypermethylation in cadmium-exposed cells (Benbrahim-Tallaa et al., 2007; Wang et al., 2012; Yuan et al., 2013). Therefore, in order to elucidate the mechanisms underlying cadmium-induced carcinogenesis, it is important to investigate the cadmium-induced carcinogenesis by focusing on the epigenetic changes.

Apolipoprotein E (ApoE) is a key gene for the regulation of lipid metabolism and cholesterol homeostasis (Mahley, 1988). ApoE has a liver X receptor (LXR) response element (LXRE) in its promoter region (Laffitte et al., 2001; Lu et al., 2009; Yue and Mazzone, 2009), and ApoE expression is directly regulated by LXRα (Ulven et al., 2004; Lu et al., 2009). Recently, it has been demonstrated that ApoE molecule is a suppressor for cell invasion as a novel physiological function (Bhattacharjee et al., 2011; Pencheva et al., 2012). In general, cell proliferation, invasion, and migration are increased as cells undergo malignant transformation. This is true for cells malignantly transformed by cadmium (Takiguchi et al., 2003). Thus, if ApoE plays an important role in control of cell invasion it is possible that ApoE activity could be altered during cadmium-induced hyper-invasiveness associated with malignant transformation. Any such altered ApoE expression could be from epigenetic modification of the ApoE gene in cadmium-exposed cells.

The rat liver epithelial cell line TRL 1215 (TRL 1215 cells) has been widely used as an *in vitro* model for metal-induced malignant transformation (Zhao et al., 1997). We have previously shown that malignant transformation in TRL 1215 cells is induced by exposure to 2.5 μM cadmium for 10 weeks (Takiguchi et al., 2003). In addition, these cadmium-induced malignant transformants show persistent genomic DNA hypermethylation despite placing cells in medium freed from cadmium for up to an additional 4 weeks (Takiguchi et al., 2003), implicating cadmium-associated epigenetic changes in concert with transformation. These cells also show the hallmarks of acquired malignancy with cadmium treatment, including greatly increased invasion (Takiguchi et al., 2003). Given this aspect of increased invasion together with the persistent epigenetic changes in seen cadmium transformed TRL 1215 cells we suspected genes involved with cell invasion and susceptible to epigenetic control could be a factor, Thus, in the present study, we focused on the ApoE in the malignant transformation induced by cadmium and investigated whether ApoE expression is epigenetically regulated by cadmium in TRL 1215 cells, to reveal any relationship between epigenetic changes on ApoE expression and stimulated cell invasion during cadmium-induced malignant transformation.

2. Materials and methods

2.1. Reagents

5-Aza-2′-deoxycytidine (5-aza-dC) and LXR agonist GW3965 were purchased from Sigma-Aldrich (St. Louis, MO, USA) and Santa Cruz Biotechnology (Santa Cruz, CA, USA), respectively. Anti-ApoE antibody and anti-actin antibody were purchased from Santa Cruz Biotechnology and Sigma-Aldrich, respectively. Anti-goat IgG antibody and anti-rabbit antibody were purchased from Sigma-Aldrich and Vector Laboratories (Burlingame, CA, USA), respectively.

2.2. Cell cultures and treatments

The TRL1215 cells were originally derived from neonatal rat liver that was finely minced, untreated and cultured as described (Idoine et al., 1976). The cell line arose spontaneously and can be subjected to long-term culture while maintaining hepatocyte features but without acquiring a malignant phenotype (spontaneous immortalization). The cells were cultured in RPMI 1640 (Sigma-Aldrich) supplemented with heat inactivated 10% fetal bovine serum (FBS) (Equitech-Bio, Kerrville, TX, USA), under an atmosphere of 5% CO_2 at 37 °C. TRL 1215 cells were exposed to 2.5 μM cadmium chloride for 10 weeks, and then the cells were cultured in cadmium-free medium for 4 weeks (Fig. 1A).

2.3. Cell viability analysis (MTS assay)

In the cell viability study (Fig. 1C), cells were seeded into 96-well plates at a density of ~5000 cells/well. After 48 h of

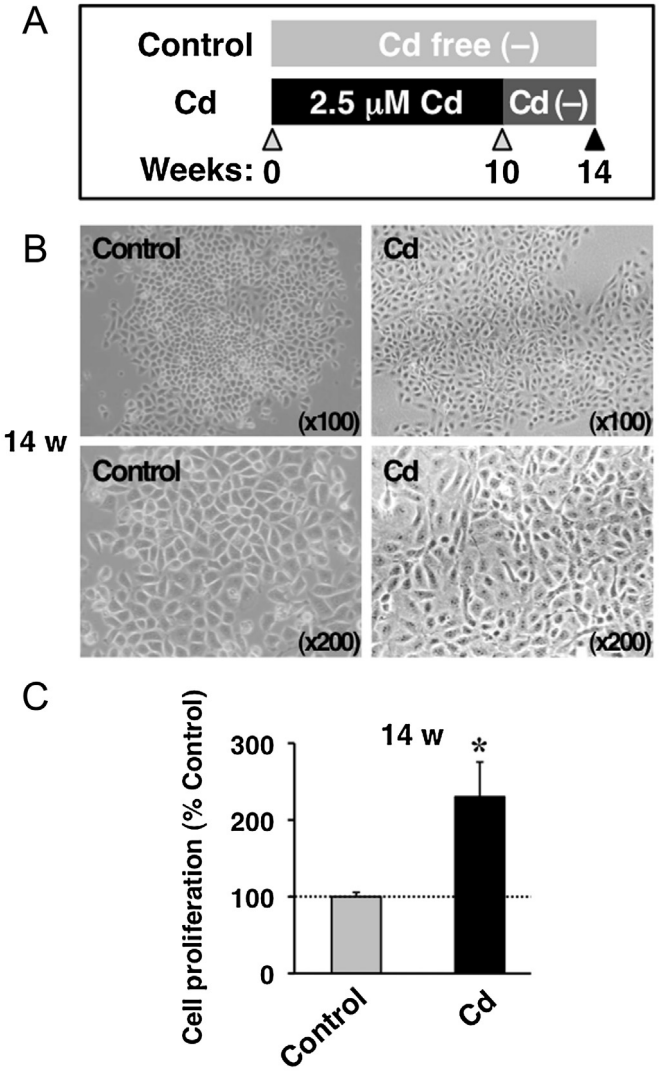

Fig. 1. Evidence of transformation of liver TRL 1215 cells by chronic cadmium exposure. (A) Method for treatment with cadmium. TRL 1215 cells chronically exposed to 2.5 μM cadmium for 10 weeks, followed by placing cadmium-free medium for an additional 4 weeks. (B) Effects of chronic exposure to cadmium on the morphology of TRL 1215 cells. (C) Effects of chronic exposure to cadmium on cell proliferation. Data are expressed as the percent of the passage-matched control (control), and represent the mean ± S.E. ($n = 3$). *Significantly different ($P < 0.05$) from the passage-matched control.