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S100P is a potential molecular target of cadmium-induced inhibition of human placental trophoblast cell proliferation

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ABSTRACT

Cadmium, a common and highly toxic pollutant, has been known to accumulate high concentrations in placenta with deleterious effects on placental structure and function. Cadmium inhibits cell proliferation in placenta via targeting metal binding proteins. S100P, a Ca^{2+}-binding protein, plays an important role in promoting cell proliferation and our previous study found its downregulation was linked to cadmium exposure in Guiyu, a famous e-waste recycling town in China. So, the present study was aimed to define whether cadmium inhibited cell proliferation through interfering with S100P. Using human trophoblast-derived HTR-8/SVneo cells as a model in vitro, we showed that cadmium exposure led to decreases in both cell proliferation and S100P expression. Knockdown of S100P in HTR-8/SVneo cells led to an obvious decrease of cell proliferation, and upregulation of S100P resulted in a significant increase of cell proliferation. Furthermore, after 24 h of exposure to cadmium (20 μM), cells transfected with pcDNA3.1-S100P showed a 1.3-fold higher S100P protein level, 38% higher proliferation evaluated with MTT assay than cells with no transfection, indicating that S100P expression attenuated cadmium-induced inhibition of cell proliferation. Taken together, we demonstrate that cadmium inhibits S100P expression and cell proliferation in placenta, meanwhile, S100P expression affects cell proliferation. Thus, our study is the first to indicate that cadmium may induce inhibition of placental trophoblast cell proliferation through targeting S100P.

1. Introduction

Cadmium is a widespread environmental toxicant releasing from industrial and agricultural waste (Faroon et al., 2012). Exposure to Cadmium for the general population occurs mainly through diet, work and tabacco smoking (Jarup and Akesson, 2009). It has been identified that cadmium can accumulate in many organs, including kidney, liver, bone and nervous system, and result in adverse effects on these organs (Thevenod and Lee, 2013). Additionally, the placenta is a major target of cadmium in pregnant women for its barrier function against the transfer of heavy metals to the fetus (Myllynen et al., 2005). Cadmium can accumulate high concentrations in placenta (Esteban-Vasallo et al., 2012) and prenatal exposure to cadmium can induce both structural and functional abnormalities of placenta (Baranski et al., 1982; Di Sant'Agnese et al., 1983). It's reported that cadmium exposure may lead to implantation delay, premature delivery, placental necrosis and early pregnancy loss by mechanisms such as oxidative stress, endocrine disorders and altered ion transportation (Kawai et al., 2002; Kippler et al., 2010, 2012; Levin et al., 1981; Lin et al., 1997; Stasenko et al., 2010). In addition, inhibition of cell proliferation has been suggested to be a potential mechanism involved in cadmium-induced impairment on placental development (Piersma et al., 1993; Powlin et al., 1997; Thompson and Bannigan, 2008). However, it is poorly known about the molecular targets of cadmium toxicity in placenta.

Cadmium treatment was proved to decrease cytosolic Ca^{2+} binding activities and downregulate the expression of a Ca^{2+}-binding protein, HCaBP of trophoblastic cells (Lin et al., 1997), suggesting that the exposure to cadmium can deregulate the expression of Ca^{2+}-binding proteins. Among the Ca^{2+}-binding proteins, S100P, a member of the Ca^{2+}-binding S100 protein family, attracts our attention. S100P was originally purified from the placenta and strongly expressed in trophoblast cells (Becker et al.,

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Table 1
Primers for real-time PCR.

	Primer sequence(5′ to 3′)	Size(bp)	Annealing Tm(°C)
S100P	F:GGAGAAGGAGCTACCAGG R: GCCACGAACACGATGAAC	126	60
β-actin	F: AGCGAGCATCCCCCAAAGTT R: GGGCACGAAGGCTCATCATT	285	60

F: forward. R: reverse.

1992; Parkkila et al., 2008). Though little is known about its precise function in placenta, it's important roles in tumor progression in promoting cellular proliferation and enhancing survival in different tumor cell types have been well studied (Arumugam et al., 2004; Tothova and Gibadulinova, 2013). Recently, a study reported that S100P can also regulate the proliferation of trophoblast-like cells, showing it's potential role in pregnant development (Zhu et al., 2015). Furthermore, our previous study found that placentas from women living in Guiyu, a major processing center for electronic waste, showed lower S100P levels compared with placentas from women living in Shantou, an area without electronic waste pollution, moreover, the downregulation of S100P was associated with cadmium exposure (Zhang et al., 2011). This finding suggests S100P may be a molecular target of heavy metals, especially cadmium in placenta.

As mentioned above, we hypothesized that cadmium might induce the deregulation of cell proliferation in placenta partly through targeting S100P. In the present study, we examined this hypothesis using human extravillous trophoblast-derived HTR-8/SVneo cells as a model in vitro. We evaluated the effects of cadmium exposure on S100P expression and cell proliferation separately at different concentrations. Then we tested the effect of S100P expression on cell preliferation. Finally, we investigated the influence of increased S100P level on cell proliferation under cadmium exposure.

2. Materials and methods

2.1. Cell culture

The human trophoblast-derived HTR-8/SVneo cells were purchased from Jenniobio Biotechnology (Guangzhou, China). The cell line was cultured in RPMI 1640 medium (Life Technologies, New York, USA) supplemented with 10% fetal bovine serum and incubated at 37 °C with 5% CO_2.

2.2. MTT assay

HTR-8/SVneo cells were seeded in 96-well plates at density of 1×10^4 cells/well. After being 80% confluent, Cells were treated with Cadmium chloride ($CdCl_2$) (Sigma, USA) for 24 h at the doses of 2 and 20 μM. $CdCl_2$ solutions were prepared using dimethyl sulfoxide (DMSO, sigma, USA) as described by Radio et al. (2008), and the final DMSO concentration in the culture media was 0.1%. In the vehicle control, cells were treat with 0.1% DMSO only. After incubation, cell proliferation was assessed by MTT assay with 3 repeated wells.

In MTT assay, 20 μl of 5 mg/ml MTT solution was added in each well, and the plate was incubated for 4 h at 37 °C. The medium was then removed and the colored reaction product was solubilized in

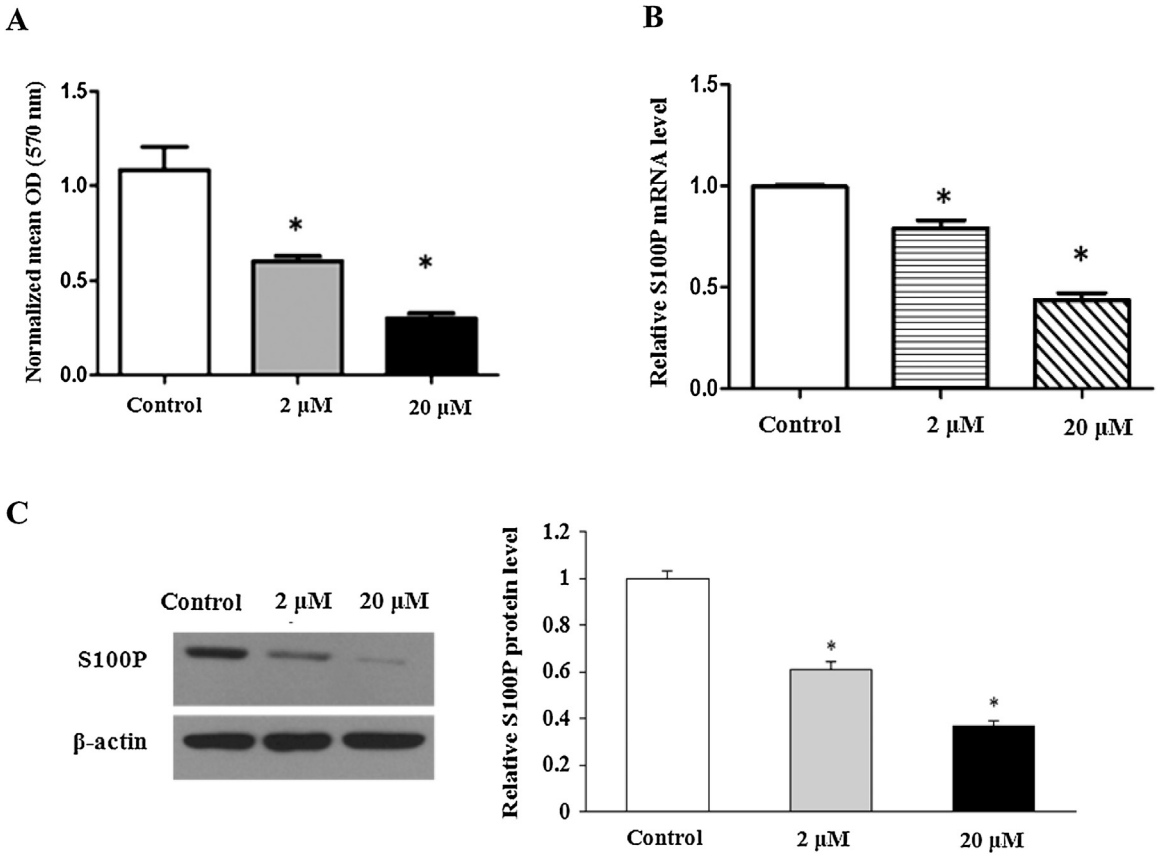

Fig. 1. Effects of $CdCl_2$ on proliferation of HTR-8/SVneo cells and S100P expression. A. Cell proliferation was determined by MTT assay. Cells were treated with 2 and 20 μM $CdCl_2$ for 24 h. As a contol, $CdCl_2$ was replaced with DMSO. B. Relative mRNA levels of S100P by real-time PCR. C. Protein levels of S100P by western blot anaysis. The band intensities were quantitated using ImageJ software. The results were normalieze with β-actin and expressed as fold change relative to control. Data are represented as mean ± SEM of at least 3 independent experiments. $^*P < 0.05$ vs. vehicle control.