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Roles of ROS, Nrf2, and autophagy in cadmium-carcinogenesis and its prevention by sulforaphane

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ABSTRACT

Environmental and occupational exposures to cadmium increase the risk of various cancers, including lung cancer. The carcinogenic mechanism of cadmium, including its prevention remains to be investigated. Using fluorescence and electron spin resonance spin trapping, the present study shows that in immortalized lung cells (BEAS-2BR cells), exposure cadmium generated reactive oxygen species (ROS). Through ROS generation, cadmium increased the protein level of TNF-α, which activated NF-κB and its target protein COX-2, creating an inflammatory microenvironment. As measured by anchorage-independent colony formation assay, cadmium induced malignant cell transformation. Inhibition of ROS by antioxidants inhibited transformation, showing that ROS were important in the mechanism of this process. The inflammatory microenvironment created by cadmium may also contribute to the mechanism of the transformation. Using tandem fluorescence protein mCherry-GFP-LC3 construct, the present study shows that cadmium-transformed cells had a property of autophagy deficiency, resulting in accumulation of autophagosomes and increased p62. This protein upregulated Nrf2, which also upregulated p62 through positive feed-back mechanism. Constitutive Nrf2 activation increased its downstream anti-apoptotic proteins, Bcl-2 and Bcl-xl, resulting in apoptosis resistance. In untransformed BEAS-2BR cells, sulforaphane, a natural compound, increased autophagy, activated Nrf2, and decreased ROS. In cadmium-transformed BEAS-2BR cells, sulforaphane restored autophagy, decreased Nrf2, and decreased apoptosis resistance. In untransformed cells, this sulforaphane induced inducible Nrf2 to decrease ROS and possibly malignant cell transformation. In cadmium-transformed cells, it decreased constitutive Nrf2 and reduced apoptosis resistance. The dual roles of sulforaphane make this natural compound a valuable agent for prevention against cadmium-induced carcinogenesis.

1. Introduction

Cadmium, a toxic heavy metal, is classified as a known human carcinogen (IARC, 1993). The major sources of cadmium exposures are food, cigarette smoking, and cadmium related industry, such as electroplating, pigment, and batteries (Rafati Rahimzadeh et al., 2017). Environmental and occupational exposures to cadmium cause inflammation and cancers of various organs, including cancer of the lung (Chen et al., 2015; Chen et al., 2016a; Chen et al., 2016b; Kim et al., 2017; Larsson et al., 2015). Although the mechanism of cadmium-induced carcinogenesis remains to be defined, ROS are considered the important mechanism in cadmium-induced carcinogenesis (Wang et al., 2016). ROS induce intracellular oxidative stress, which could damage macromolecules and eventually contribute to a variety of diseases including cancer (Wang et al., 2016). While carcinogenesis is a multiple step process, when discussing the known mechanisms of metal-induced carcinogenesis, we conceptually refer to two stages. In the first stage of cadmium-induced carcinogenesis (from normal cells to transformed cells), ROS play a major role in the malignant cells transformation of BEAS-2BR cells exposed to cadmium (Son et al., 2012; Xu et al., 2017). Inhibition of ROS using antioxidant [catalase (CAT) or superoxide dismutase (SOD)] is able to decrease cadmium-induced carcinogenesis (Son et al., 2012). Although the mechanism of the first stage of metal carcinogenesis is very extensively studied, the mechanism of the second

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stage of metal carcinogenesis (morphologically transformed cells progress into tumorigenesis) is not very well investigated. Our previous study (Son et al., 2014) showed that in cadmium-transformed cells, p62 and Nrf2 were constitutively activated and their downstream antioxidants and anti-apoptotic proteins were elevated. The final outcomes are a decrease in ROS, apoptosis resistance, and tumorigenesis (Son et al., 2014). A decrease of ROS generation in the second stage of metal-induced carcinogenesis is oncogenic, because it provides a favorable environment for the survival and tumorigenesis of transformed cells (Wang et al., 2016; Xu et al., 2017). Thus, a decrease of ROS generation in the first stage of cadmium carcinogenesis and upregulation of ROS generation in the second stage could be a strategy to inhibit cadmium induced carcinogenesis.

Persistent inflammation contributes to carcinogenesis and tumor progression by activating a series of inflammatory molecules and a creation of an inflammatory tumor microenvironment favorable for cancer growth (Sui et al., 2017). One of the pro-inflammatory cytokines, tumor necrosis factor alpha (TNF-α), activates cancer cell survival and proliferation pathway, triggers inflammatory cell infiltration of tumor, and promotes angiogenesis and tumor cell migration and invasion (Balkwill, 2006). TNF-α activates NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) pathway, which is important in carcinogenesis (Wu and Zhou, 2010). Activation of Cyclooxygenase-2 (COX-2) generates an inflammatory microenvironment, which is important for early-stage tumorigenesis (Echizen et al., 2018). Although, cadmium is able to induce inflammation, which is known to be involved in cancer initiation and progression (Kim et al., 2017; Olszowski et al., 2012; Phuagkhaopong et al., 2017). The role of inflammation in cadmium-induced carcinogenesis remains to be determined.

The role of autophagy in the mechanism of metal carcinogenesis is increasingly recognized. Autophagy is a self-degradative process and plays a housekeeping role in removing proteins, clearing damaged organelles, and eliminating intracellular pathogens (Glick et al., 2010). Owning to a key role in the preservation of intracellular homeostasis, autophagy constitutes a barrier against various degenerative processes that may affect healthy cells, including carcinogenesis (Chen and Karantza, 2011; Levine and Klionsky, 2004). Our previous studies showed that autophagy inhibited arsenic-induced ROS generation by facilitating mitochondrial turnover in untransformed BEAS-2BR cells (Zhang et al., 2012). Thus, autophagy can exhibit anti-oncogenic effects. During the autophagy process, cytosolic substrates are assimilated in double-membrane vesicles or autophagosome, and subsequently transferred to lysosome to be degraded and recycled in autolysosome (the fusion product of autophagosome and lysosome) (Nakahira et al., 2014). Cadmium treatment increased autophagy in untransformed BEAS-2BR cells, while cadmium-transformed cells are autophagy-deficient, which is a blockage of the fusion between autophagosomes and lysosomes to prevent autophagy from completion (Son et al., 2014). In autophagy competent cells, p62 binds directly to LC3 to facilitate degradation of p62 and ubiquitinated protein aggregates by autolysosomes (Pankiv et al., 2007). In autophagy deficiency cells, the inability of the cells to remove p62 causes accumulation of p62 (Son et al., 2014), which is oncogenic. Thus, the investigation of autophagy deficiency in cadmium-transformed cells will provide important information for understanding the mechanism of second stage of cadmium carcinogenesis.

In cadmium-transformed cells, high expression of p62 due to autophagy deficiency causes p62 to bind to Keap 1 (kelch-like ECH-associated protein 1), the inhibitor of Nrf2 (nuclear factor erythroid 2-related factor), leading to constitutive activation of Nrf2. Constitutively activated Nrf2 is translocated from cytosol to nucleus, where it binds to antioxidant response elements (ARE) in the promoter regions of Nrf2 downstream proteins, such as superoxide dismutase, catalase and anti-apoptotic proteins such as Bcl2 and Bcl-xl. The final results are the decrease in ROS, development of apoptosis resistance, and creation of a favorable environment for tumorigenesis of cadmium-transformed cells (Son et al., 2014). In addition, Nrf2 also upregulates p62 and has a positive feedback by binding directly to the ARE site of p62 (Jain et al., 2010). Nrf2 is likely to act as a double-edged sword in metal induced-carcinogenesis. In the first stage of metal carcinogenesis, the antioxidant property of Nrf2 decreases ROS to protect cells from malignant transformation. In the second stage of metal carcinogenesis, constitutive activation of Nrf2 leads to decrease in ROS generation and acquired apoptosis resistance, providing a favorable environment for the survival and tumorigenesis of transformed cells (Xu et al., 2017).

Sulforaphane (SFN), a natural dietary isothiocyanate produced by cruciferous vegetable such as broccoli, displays a very strong anti-cancer, anti-oxidant, and anti-inflammatory activities (Brandenburg et al., 2010; Sestili and Fimognari, 2015). Due to safety, efficiency, and practicability, foods containing bioactive phytochemicals are gaining significant attention as elements of chemoprevention strategies against cancer (Yang et al., 2016a). Previous studies indicate that sulforaphane reduced cadmium-induced toxic effects in different cells (Alkharashi et al., 2017; Alkharashi et al., 2018; Yang et al., 2016b). As potent naturally occurring inducers of Nrf2 signaling, sulforaphane exhibits its cytoprotective property through Nrf2 pathway (Yang et al., 2016a). Since in the first stage of cadmium carcinogenesis Nrf2 is anti-oncogenic, we hypothesized that induction of Nrf2 by sulforaphane decreased cadmium-generated ROS, leading to inhibition of cadmium-induced malignant transformation of BEAS-2BR cells. In the second stage of cadmium carcinogenesis, Nrf2 is constitutively activated through accumulation of p62 and lost its inducibility due to autophagy deficiency, leading to apoptosis resistance and tumorigenesis of cadmium-transformed cells. Previous studies also reported that sulforaphane increased autophagy flux in certain cells (Herman-Antosiewicz et al., 2006; Lee et al., 2014). Thus, it is very likely that sulforaphane may activate inducible Nrf2 in normal cells to reduce ROS and to decrease malignant cell transformation and that in cadmium-transformed cells sulforaphane may restore autophagy competence, leading to decrease of p62/Nrf2, apoptosis resistance and tumorigenesis of cadmium-transformed cells.

2. Materials and methods

2.1. Chemicals and cultureware

Unless specified otherwise all chemicals and culturewares were purchased from Sigma Chemical Co. (St Louis, Mo) and Falcon Labware (Bectone-Dickinson, Franklin Lakes, NJ), respectively. Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), and antibiotic-antimycotic were purchased from Gibco Company (Gibco BRL, NY).

2.2. Cell lines and cell culture

The human bronchial epithelial cell line (BEAS-2BR cells) was obtained from the American Type Culture Collection and cadmium-transformed cells were generated as described previously (Son et al., 2014). Cadmium-transformed cells and their passage-matched untransformed BEAS-2BR cells were maintained in DMEM supplemented with 10% fetal bovine serum and 1% antibiotic-antimycotic. When grown with serum, BEAS-2B cells become serum resistant and lose their ability to differentiate, thus we refer to them as BEAS-2BR cells when grow in a serum system to signify that they are serum resistant BEAS-2B cells (Ke et al., 1988).

2.3. Plasmids, shRNA, and transfection

Overexpression of catalase, SOD1 and SOD2 were performed as described previously (Son et al., 2012). Nrf2 and p62 shRNA were purchased from Origene (Rockville, MD). Transfections were performed using Lipofectamine™ 2000 (Invitrogen) according to the