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Reductive detoxification of acrolein as a potential role for aldehyde reductase (AKR1A) in mammals

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ABSTRACT

Aldehyde reductase (AKR1A), a member of the aldo–keto reductase superfamily, suppresses diabetic complications via a reduction in metabolic intermediates; it also plays a role in ascorbic acid biosynthesis in mice. Because primates cannot synthesize ascorbic acid, a principle role of AKR1A appears to be the reductive detoxification of aldehydes. In this study, we isolated and immortalized mouse embryonic fibroblasts (MEFs) from wild-type (WT) and human *Akr1a*-transgenic (Tg) mice and used them to investigate the potential roles of AKR1A under culture conditions. Tg MEFs showed higher methylglyoxal- and acrolein-reducing activities than WT MEFs and also were more resistant to cytotoxicity. Enzymatic analyses of purified rat AKR1A showed that the efficiency of the acrolein reduction was about 20% that of glyceraldehyde. Ascorbic acid levels were quite low in the MEFs, and while the administration of ascorbic acid to the cells increased the intracellular levels of ascorbic acid, it had no affect on the resistance to acrolein. Endoplasmic reticulum stress and protein carbonylation induced by acrolein treatment were less evident in Tg MEFs than in WT MEFs. These data collectively indicate that one of the principle roles of AKR1A in primates is the reductive detoxification of aldehydes, notably acrolein, and protection from its detrimental effects.

1. Introduction

Unsaturated fatty acids are prone to oxidation by reactive oxygen species (ROS), and the resultant lipid peroxidation products mostly contain aldehyde moieties. Among lipid-derived aldehydes, 4-hydroxy 2-nonenal (HNE) and malondialdehyde are abundantly produced as peroxidation products [1]. While acrolein (2-propenal) is produced to a lesser extent under oxidative conditions, it also comes mostly from tobacco smoke, heated cooking oil, and air pollutants, and it exerts the strongest cytotoxicity from among the lipid-derived aldehydes [2]. Acrolein is a major environmental risk factor for chronic obstructive pulmonary disease (COPD), and augmented levels of acrolein are found in the lung fluids of COPD patients [3]. The cytotoxic effects of acrolein are mediated by the modification of a variety of molecules including proteins and nucleic acids [4–6], which impairs glutathione homeostasis [7–9]. The formation of a DNA adduct with acrolein leads to mutagenesis and ultimately to tumorigenesis [10]. Recent studies have indicated that acrolein exerts its cytotoxicity by triggering mitochondrial damage and endoplasmic reticulum (ER) stress in pulmonary cells [11] and endothelial cells [12], in the liver [13], and in other cell lines [14]. Thus, it is intriguing to learn how acrolein can be detoxified *in vivo* from the viewpoint of health maintenance.

Enzymes in the aldo–keto reductase (AKR) superfamily catalyze the reduction of various aldehydes to their corresponding alcohols in an NADPH-dependent manner [15]. Some aldo–keto reductase family members such as aldose reductase (AKR1B), AKR1B7, AKR1C, aflatoxin B1 aldehyde reductase (AKR7A1), and AKR7A2 are reportedly involved in the detoxification of acrolein [16–21]. However, their abundance is limited in the liver, a main detoxification organ. Aldehyde reductase (AKR1A), which is highly expressed and more abundant than these family members in the liver, suppresses diabetic complications via a reduction of 3-deoxyglucosone and methylglyoxal, which are intermediates of the glycation reaction and are cytotoxic [22,23]. AKR1A is also involved in some metabolic pathways that require a reduction of the aldehyde moieties of intermediary compounds; e.g., the

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conversion of prostaglandin H_2 to prostaglandin F_2 [24]. Recently, Gabbay et al. [25] found that AKR1A and AKR1B are the enzymes responsible for the production of ascorbic acid (AsA). Although the two isozymes catalyze the same D-glucuronic acid reducing reaction process, the contribution of AKR1A to the AsA synthetic pathway is dominant due to its abundance in the liver, a primary organ in the AsA supply. Hence, AKR1A-deficient mice show severe osteopenia and spontaneous fractures due to AsA deficiency, while AKR1B-deficient mice do not. We have independently established an *Akrla* knockout mouse, along with human *Akr1a*-trangenic (Tg) mice, and demonstrated that AKR1A catalyzes the reduction of D-glucuronic acid and D-glucuronio-γ−lactone during AsA biosynthesis [26].

However, the role of AKR1A in acrolein detoxification is unclear despite the abundant expression of AKR1A in the liver [22]. Due to the broad substrate specificity, and to similarities in the enzyme characteristics, it is difficult to distinguish the individual roles of the AKR family enzymes *in vivo*. In the present study, we investigated embryonic fibroblasts (MEFs) from *Akrla*-gene modified mice as well as wild-type mice from the view-point of aldehyde toxicity and demonstrated a conceptual role of AKR1A in primates—that of acrolein detoxification.

2. Materials and methods

2.1. Chemicals

D-glucuronic acid and methylglyoxal were purchased from Sigma–Aldrich (St. Louis, MO). Acrolein was purchased from Tokyo Chemical Industry (Tokyo, Japan). D,L-glyceraldehyde was acquired from Nacalai Tesque (Kyoto, Japan). L-ascorbic acid and all other reagents were obtained from Wako Pure Chemical (Osaka, Japan).

2.2. Preparation of MEFs

E13.5 embryos from WT or Tg mice established in the previous study [26] were used for the preparation of MEFs. The head, four limbs and tail of each embryo were removed and kept for genotyping. After removal of the visceral organs, the remainder of the body was minced finely and digested with 0.1% trypsin in phosphate-buffered saline (PBS) at 37 °C for 15 min. Cells were dispersed and suspended in 10 ml of complete medium (DMEM, 100 units penicillin, 0.1 mg/ml streptomycin, 10% fetal bovine serum). The resultant cell suspension was transferred to plastic dishes and incubated in a CO_2 incubator at 37 °C.

2.3. Immortalization of MEFs by SV-40 large T antigen

Retroviruses were produced in a Phoenix helper cell line transfected with pBABE-puro SV-40 large T antigen vector (Addgene). WT and human *Akr1a*-Tg MEFs were immortalized in medium containing the retrovirus and 4 μg/ml of Polybrene (Millipore). The immortalized cells were selected by culture medium with 2.5 μg/ml of puromycin.

2.4. Cellular viability assay

MEFs grown to 80% were exposed to each aldehyde compound and cultured for 24 h. After washing with PBS, attached cells were stained with 0.1% crystal violet/1% methanol in PBS for 20 min and washed 4 times with water. The stain was solubilized in 0.5% (w/v) SDS and absorbance was recoded at $\lambda = 590$ nm in a microplate reader (Valioskan Flash, Thermo Fisher Scientific).

2.5. Protein preparation

MEFs were rinsed twice and harvested with ice-cold PBS. After centrifugation, cell pellets were lysed in RIPA buffer (50 mM Tris–HCl, pH 8.0, 150 mM NaCl, 1% (w/v) Nonidet P-40, 0.5% (w/v) Deoxycholate, 0.1% SDS) containing 50 mM NaF, 2.5 mM Na-pyrophosphate, 2 mM sodium orthovanadate, 25 mM β-glycerophosphate, 40 μM APMSF, and protease inhibitor cocktail (Roche) and centrifuged at 17,400×*g* for 10 min at 4 °C. The supernatant were subjected to protein determination using a BCA kit (Pierce) followed by immunoblot analyses. For the cellular ascorbic acid and enzyme assay, cell pellets were lysed in hypotonic buffer (25 mM Hepes, pH7.5, 1 mM EDTA) with brief sonication (Microson, Misonix Inc.).

2.6. Immunoblot analyses

Aliquots of protein (40–60 μg) were separated on 7.5% or 10% SDS–polyacrylamide gels and electroblotted onto polyvinylidene difluoride (PVDF) membranes (GE Healthcare). The blots were blocked with 3% skim milk in Tris-buffered saline containing 0.1% Tween-20 (TBST), and were then incubated with the polyclonal antibodies against AKR1A [22], AKR1B [27], SV40 large T antigen (sc-148, Santa Cruz), IRE1α (#3294, Cell Signaling), phosphorylated IRE1α (p-IRE1α, NB100-2323, NOVUS), CHOP (sc-7351, Santa Cruz), BiP (sc-13968, Santa Cruz), and the mouse monoclonal antibody against β-actin (sc-69879, Santa Cruz) diluted in TBST containing 1% BSA. After three washes in TBST, the blots were incubated with horseradish peroxidase-conjugated goat anti-rabbit or anti-mouse IgG antibody (Santa Cruz). After washing, the presence of bound horseradish peroxidase was detected by measuring chemiluminescence using Immobilon western chemiluminescent HRP substrate (Millipore) on an image analyzer (ImageQuant LAS500, GE Healthcare). A carbonyl blot was performed using an OxyBlot kit (Millipore) according to the manufacturer's instruction.

2.7. Measurement of cellular ascorbic acid

We synthesized 15-(Naphthalen-1-ylamino)-7-aza-3, 11-dioxadispiro[5.1.5^8.3^6]hexadecan-7-oxyl (Naph-DiPy) [28], for use as a fluorescent probe to measure the AsA in the cells, as described [29]. The cell lysate was incubated with Naph-DiPy for 30 min in RT. The AsA concentration was calculated by measuring the fluorescence at an excitation wavelength of 310 nm and at an emission

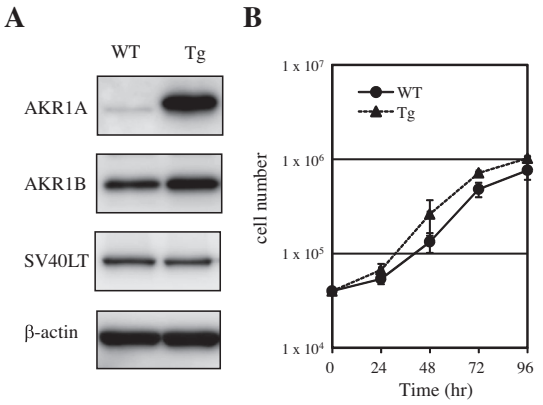

Fig. 1. Characteristics of immortalized MEFs. (A) Representative data from the immunoblots of soluble proteins from the immortalized WT and Tg MEFs. Representative data of several experiments are shown. (B) The number of cells cultivated in 3.5 cm dishes was counted at corresponding time points and is expressed per dish ($n = 3$).