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Decreased levels of *AKR1B1* and *AKR1B10* in cancerous endometrium compared to adjacent non-cancerous tissue

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ABSTRACT

Endometrial cancer is associated with enhanced cell proliferation due to high concentrations of estrogens, and decreased cell differentiation due to low levels of progesterone and retinoic acid. It is also associated with aberrant inflammatory responses and concomitant increased production of prostaglandins. The human members of the aldo–keto reductase 1B (AKR1B) subfamily, AKR1B1 and AKR1B10, have roles in these processes and can thus be implicated in endometrial cancer. To date, there have been no reports on the expression of AKR1B1 in endometrial cancer, while AKR1B10 has only been studied at the cellular level. To evaluate the roles of these AKR1B enzymes, we investigated expression of *AKR1B1* and *AKR1B10* in 47 paired samples of cancerous and adjacent control endometrium at the mRNA and protein levels, by quantitative PCR, Western blotting and immunohistochemistry staining. There were significantly lower mRNA and protein levels of *AKR1B1* in cancerous tissues compared to adjacent endometrium. The gene expression of *AKR1B10* at the mRNA level was significantly increased, while there were significantly decreased protein levels. Immunohistochemistry revealed that both of these enzymes were present in all of the samples, and are located in epithelial cells of cancerous and control endometrial glands. Elevated levels in adjacent non-cancerous tissues imply that these enzymes are more important in the initiation of endometrial cancer than in its progression. To the best of our knowledge, this is the first report on the expression of *AKR1B1* and *AKR1B10* in endometrial cancer. Further studies are needed to define the precise roles of these enzymes in the pathogenesis of endometrial cancer.

1. Introduction

Endometrial cancer (EC) is the most common gynecological malignancy in the western world [1,2]. The majority of ECs are sporadic and can be divided into two subgroups [2,3]. The first is type I, or estrogen-dependent, endometrioid endometrial carcinoma, which is usually low-grade. This EC represents the majority of cases (80%) and arises on a background of endometrial hyperplasia [4]. The second EC is type II, or non-endometrioid, endometrial carcinoma, and this accounts for 20% of cases. It is typically high grade, and is believed to follow an estrogen-unrelated pathway [5]. Some tumors share the pathological and molecular features of types I and II ECs, and so they fall into the gray zone that shows overlapping clinical, morphological, immunohistochemical, and molecular features [6].

Development of EC is associated with a number of risk factors, such as obesity, nulliparity, early age at menarche, and late onset of menopause [7]. It is also related to decreased cell differentiation, due to the low levels of protective progesterone and retinoic acid, and to enhanced cell proliferation, due to high local concentrations of estrogens [5,8,9]. Furthermore, the involvement of inflammation in the pathogenesis of EC via its mediators, the prostaglandins (PGs), has been suggested recently [7]. Locally formed PGE2 and PGF2α can stimulate cell proliferation, adhesion and migration, and angiogenesis in cancerous endometrium [7,10,11]. The aldo–keto reductases 1B (AKR1B) have roles in cell proliferation, differentiation and inflammation and can thus be implicated in the development of EC.

There are three human members of the AKR1B subfamily: AKR1B1, AKR1B10 and AKR1B15 [12]. To date, only AKR1B1 and AKR1B10 are well-characterized. They are known as aldose reductases, and they catalyze the reduction of aldehydes to alcohols, with the reduction of a series of other substrates [13–15]. The physiological function and substrates of human AKR1B15 have not yet been determined [12].

AKR1B1, which is also known as aldose reductase, is a ubiquitously expressed enzyme that regulates the polyol pathway of glu-

Abbreviations: AKR1B1, aldo–keto reductase 1B1; AKR1B10, aldo–keto reductase 1B10.

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cose metabolism and lipid-aldehyde-mediated cell signaling (Fig. 1) [16,17]. As AKR1B1 catalyzes the reduction of glucose to sorbitol it is related to the development of tissue injury associated with diabetes [18]. Additionally, AKR1B1 can catalyze the reduction of lipid peroxidation products, and with higher catalytic efficiency, their glutathione (GSH) conjugates [15,17]. Lipid aldehydes alter cell signals through their regulation of the activation of transcription factor NFκB; therefore, by reducing GSH-aldehydes, AKR1B1 can indirectly stimulate NFκB and activate inflammatory processes [16,17]. AKR1B1 is also a functional PGF2α synthase [19], and it converts PGH2 into PGF2α with a lower K_M and a higher V_{max} compared to AKR1C3 [20]. AKR1B1 has been implicated in the development of several human cancers (liver, breast, ovarian and cervical cancers) and also with resistance towards certain anticancer drugs [9,21].

AKR1B10 shares 71% sequence identity with AKR1B1, but differs from AKR1B1 in its tissue distribution and substrate specificity [17]. AKR1B10 is a retinaldehyde (retinal) reductase and it counteracts the formation of retinoic acid (RA) by converting retinal back to retinol (vitamin A) (Fig. 2) [9]. RA is essential for growth and maintenance of different organs and tissues, by being involved in the regulation of cell proliferation, differentiation and apoptosis [14]. In addition to retinal reduction, AKR1B10 reduces isoprenyl aldehydes, and is therefore implicated in prenylation of the small guanine nucleotide triphosphatases (GTPases) that are responsible for cell proliferation [22]. Moreover, AKR1B10 has been shown to metabolize some anticancer agents [23]. AKR1B10 is not ubiquitously expressed, although it has been detected in several cancerous tissues [24], and since low RA levels favor tumor progression, it has emerged as a tumor marker for several solid tumors [14]. It has recently been suggested, that AKR1B10 is a secretory protein and therefore a potential serum marker [25].

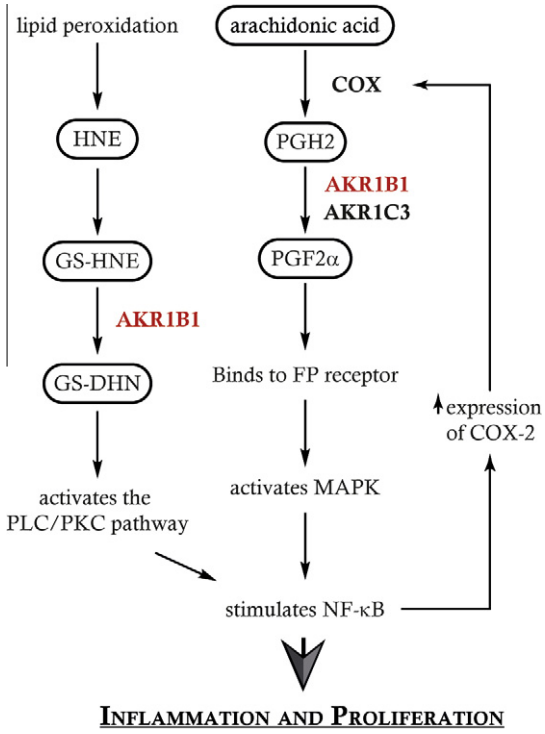

Fig. 1. Role of AKR1B1 in mediation of inflammatory signals. Cytokines, growth factors, and lipopolysaccharide can induce oxidative stress via generation of ROS leading to formation of toxic lipid aldehydes such as 4-hydroxynonenal (HNE) by lipid peroxidation. HNE conjugates with glutathione to form GS-HNE that can be reduced to GS-dihydroxynonane (GS-DHN) by AKR1B1. GS-DHN activates the phospholiase C/proteinkinase C (PLC/PKC) pathway and transcription factor NFκB which triggers the expression of inflammatory mediators such as cyclooxygenease-2 (COX-2). COX catalyzes the synthesis of PGH2 from arachidonic acid. AKR1B1 converts PGH2 into PGF2α, which acts through FP receptors, and which activates mitogen-activated protein kinase (MAPK) and NFκB. This thus leads to pathological inflammation and proliferation.

Both, AKR1B1 and AKR1B10, have been implicated in the pathogenesis of different cancers, although to date there have been no reports of the expression of AKR1B1 in ECs, while AKR1B10 has only been studied at the cellular level [9]. Therefore, we here evaluated the expression of AKR1B1 and AKR1B10 at the mRNA and protein levels in pre-menopausal and post-menopausal EC patients.

2. Materials and methods

2.1. Tissue samples

A total of 47 paired samples of EC tissues and adjacent control endometrium were collected from 16 pre-menopausal women (mean age, 46.1 ± 7.5 years) and 31 post-menopausal women (mean age, 69.5 ± 8.4 years) after hysterectomies (Table 1). The study was approved by the National Medical Ethics Committee of the Republic of Slovenia. The specimens were immediately placed into RNA Later (Qiagene, Düsseldorf, Germany), an RNA stabilization solution, and kept at −20 °C until RNA extraction. Diagnosis of EC was confirmed histologically by an experienced gynecological pathologist (J.Š.).

2.2. RNA isolation

Total RNA was isolated from the 47 paired tissue samples using Tri Reagent (Sigma–Aldrich, Saint Louis, MO, USA), according to the manufacturer instructions. The quality of the RNA samples was determined using an Agilent 2100 Bioanalyzer, and they showed an average RNA Integrity Number (RIN) of 7.5. Total RNA was reverse transcribed using SuperScript VILO cDNA Synthesis kits (Invitrogen, Carlsbad, CA, USA). The RNA samples were cleaned and the residual DNA was removed using RNeasy Mini kits and RNase-Free DNase sets (Qiagen, Düsseldorf, Germany), respectively. Samples of the total RNA (2.5 μg) were converted into cDNAs (in 50 μL), according to the manufacturer instructions, and then stored at −20 °C.

2.3. Quantitative real-time PCR

The expression of *AKR1B1*, *AKR1B10* and the three selected reference genes was examined by real-time TaqMan PCR. The most stable reference genes (*PPIA*, *HPRT1*, *POLR2A*) were selected from a cohort of 16 genes, using the GeNorm and NormFinder algorithms, as described previously [26]. The experiments were performed using the exon-spanning hydrolysis probes (FAM or VIC dye labeled) that are commercially available as 'Assay on Demand' (Applied Biosystems, Foster City, CA, USA) (Table 2). Quantification was with a LightCycler 480 Real-Time PCR system (Roche, Basel, Switzerland), using TaqMan Universal PCR Master mix and universal thermocycling parameters recommended by Applied Biosystems. Samples were run in triplicates using 0.25 μL cDNA. The reactions were performed in 384-well plates (Roche) with a reaction volume of 5 μL. The gene expression normalization factor for each sample was calculated based on the geometric mean of all three selected reference genes [27]. The PCR amplification efficiency (E) was determined from the slope of the log-linear portion of the calibration curve for each gene investigated and was accounted for in further calculations. The gene expression for each sample was calculated from quantification cycles (Cq) as E^{-Cp}, divided by the normalization factor, and multiplied by 10^{14}. The