The Biology Behind

AKR1B10: A New Diagnostic Marker of Non–Small Cell Lung Carcinoma in Smokers

□□*Commentary on Fukumoto et al., p. 1776*

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INTRODUCTION

Lung cancer is a leading cause of cancer death worldwide, and non–small cell lung carcinoma (NSCLC) accounts for 80% of the cases. Even the most innovative therapeutic regimens do not increase overall 5-year survival rates for NSCLC to >15%. Early detection methods could lead to intervention strategies that may increase these low survival rates. The article by Fukumoto et al., identifies an aldo-keto reductase (AKR) family protein, AKR1B10, as being highly over-expressed in NSCLC, suggesting that it may be a diagnostic marker. Notably, AKR1B10 was over-expressed in 84% of squamous cell carcinomas and in 29% of adenocarcinomas. In both these forms of NSCLC, over-expression of AKR1B10 was closely associated with smoking. The findings suggest that AKR1B10 is a potential diagnostic marker for smoking-related NSCLC and that it may play a role in tobacco-related carcinogenesis.

This observation raises several questions: what are AKRs? What is known about the AKR1B10 isoform? Why should AKRs be over-expressed in NSCLC? Is this an association/correlation or does it have a causal relationship?

The Aldo-Keto Reductase Superfamily

AKRs are a gene superfamily that exist from prokaryotes to eukaryotes, including man (1). They are usually monomeric (37 kDa) soluble NAD(P)H-dependent oxidoreductases that will often functionalize carbonyl groups on aldehydes or ketones to form primary or secondary alcohols. The alcohols can then be conjugated with sulfate or glucuronide for excretion. Thus, AKRs are involved in elimination reactions. AKRs belong to 14 families and the AKR1 family contains many of the human isoforms, which include the aldehyde reductases (AKR1A subfamily), the aldose reductases (AKR1B subfamily), the hydroxysteroid/dihydrodiol dehydrogenases (AKR1C subfamily), and the steroid 5β-reductases (AKR1D subfamily). Members of each family display >40% sequence identity at the amino acid level. The structural motif shared by all members of the superfamily is an $(\alpha/\beta)_8$-barrel which contains a highly conserved cofactor binding site and catalytic tetrad that is comprised of Tyr, His, Lys, and Asp. Loops at the back of the structure dictate substrate specificity.

These enzymes display broad substrate specificity and will utilize sugar and lipid aldehydes, steroid hormones, prostaglandins, and carcinogens as substrates. Their broad substrate specificity raises parallels with the CYP superfamily. Therefore, AKRs can be considered as phase I drug, xenobiotic and carcinogen-metabolizing enzymes. To learn more about the AKR superfamily, the reader is referred to the following web site: www.med.upenn.edu/akr (1, 2).

AKR1B10 Properties and Expression Patterns

AKR1B10 is related to human aldose reductase AKR1B1. AKR1B1 will covert the aldehyde on glucose to the corresponding alcohol to form sorbitol (3). The catalytic efficiency (k_{cat}/K_m) for this reaction is poor, and generally the catalytic efficiency for the reduction of other aldehydes including advanced glycation products (e.g., methylglyoxal) and lipid aldehydes (e.g., 4-hydroxy-2-nonenal) that may arise as breakdown products of lipid hydroperoxides is favored (3, 4). Based on this broad substrate specificity, it is likely that the natural substrates for AKR1B1 remain to be identified.

AKR1B10 shares 71% sequence identity with AKR1B1 (5, 6). Its catalytic efficiency for the reduction of glucose is substantially less than that observed for AKR1B1, and its preference seems to be for retinals (discussed later; ref. 4). AKR1B10 is not expressed in most human tissues. Rather, it is primarily expressed in the small intestine and colon, and a low level exists in the liver. AKR1B10 shows elevated expression in some liver cancers but it is unclear whether it contributes to the pathogenesis of the disease (6). AKR1B10 has not been shown to be expressed in normal lung, suggesting that it shows regulated expression in this organ.

Aldo-Keto Reductase Over-Expression in Non–Small Cell Lung Carcinoma

Using the technique of differential display, Hsu et al. (7) were able to show that AKR1C1 (human 20α-hydroxysteroid dehydrogenase/dihydrodiol dehydrogenase 1) was highly over-expressed >50-fold in 317 of 384 NSCLC patients. This over-expression was confirmed by immunohistochemistry and immunoblotting. In this study, tumor tissue was compared with adjacent normal tissue within the same patient. High-expression correlated with poor prognostic outcome and it was suggested that this was related to cancer chemotherapeutic drug resistance. Although a role for AKR1C1 in the metabolism of chemotherapeutic

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agents remains to be elucidated, AKR1C1 was found to be over-expressed in human ovarian carcinoma cell lines that were cisplatin resistant (8). Most, importantly when AKR1C1 was stably transfected into the parental cell line, the drug resistance phenotype was established (8). Similar to glutathione S-transferase isoforms implicated in cancer chemotherapeutic drug resistance, AKR1C1 is regulated by an antioxidant response element (9). In fact AKR1C1 was first documented as being up-regulated in ethacrynic acid-resistant human colon cells by Ciaccio and Tew (10, 11).

Up-regulation of AKR1C1 in NSCLC was confirmed by demonstrating high over-expression of the enzyme in human lung adenocarcinoma (A549) cells, using reverse transcription-PCR, immunoblot, and functional enzyme assay (12). In the present study by Fukumoto et al., seven genes were shown to be up-regulated in NSCLC, one was AKR1C1 and the other was AKR1B10, suggesting that AKR up-regulation is part of a coordinated response to cellular stressors.

AKR1B10 Up-Regulation—Potential Reasons

There are three potential reasons why AKR1B10 may be up-regulated in NSCLC: (*a*) as part of adaptive response to cellular stress, (*b*) its induction and direct involvement in carcinogen metabolism, and (*c*) its role in nuclear receptor signaling. These are not necessary mutually exclusive.

AKRs are primordial genes and respond to primordial signals. AKR1B for example is up-regulated by osmotic stress to maintain osmolarity and is regulated by an osmotic response element which is regulated by a mitogen-activated protein kinase module (13, 14). AKR1C1 is up-regulated by oxidative and electrophilic stress via the antioxidant response element which is transcriptionally regulated by Nrf-2 and small Maf (15). In each of these cases, up-regulation results in a protective response against the ensuing stressor.

AKR1C isoforms are involved in the metabolism of two classes of tobacco carcinogens, nicotine-derived nitrosamine ketone and polycyclic aromatic hydrocarbon *trans*-dihydrodiols (16, 17). In the former instance, AKR1C isoforms convert nicotine-derived nitrosamine ketone to nicotine-derived nitrosamine alcohol. Although nicotine-derived nitrosamine ketone and nicotine-derived nitrosamine alcohol can be bioactivated to the same reactive intermediates that will form O^6-methylguanine DNA-adducts, nicotine-derived nitrosamine alcohol is less genotoxic because its free secondary alcohol can be conjugated for elimination (16). In the case of polycyclic aromatic hydrocarbon *trans*-dihydrodiols, AKR1C isoforms catalyze via their dihydrodiol dehydrogenase activity the formation of reactive and redox-active polycyclic aromatic hydrocarbon *o*-quinones. These quinones have the potential to react directly with DNA to form DNA-adducts (an initiation event) or they can enter into futile redox-cycles and amplify the production of reactive oxygen species (initiation and promotion events; ref. 17). Whether AKR1B10 is induced by tobacco carcinogens or is involved in their metabolism remains to be determined.

AKR1C isoforms have also been implicated in controlling ligand access to nuclear receptors (steroid hormone receptors and PPARγ), which can lead to phenotypic responses for the target cell. AKR1C isoforms have been most studied for their 3α-, 17β-, and 20α-hydroxysteroid dehydrogenase activities where they can control levels of active androgens, estrogens, and progestins available to bind to their respective receptors (18, 19). This model for pre-receptor regulation can be expanded to other classes of ligands. AKR1C3 (3α-hydroxysteroid dehydrogenase type 2, 17β-hydroxysteroid dehydrogenase type 5 and prostaglandin F synthase) has been shown to regulate ligand access to PPARγ in human myeloid leukemia cells. In the presence of AKR1C3, prostaglandin D_2 is converted to 9α,11β-$PGF_{2\alpha}$, which leads to a proliferative response. Inhibition of this conversion by nonsteroidal anti-inflammatory drugs leads to a redirection in prostaglandin metabolism to form PGJ-2 series prostanoids that activate PPARγ, which then leads to terminal differentiation (20). Whether, AKR1B10 operates a similar differentiation/proliferation switch in human lung cancer remains to be determined. The article by Fukumoto et al. suggests that AKR1B10 is involved in the retinal-retinoic acid signaling pathway.

AKR1B10 and Retinoic Acid Signaling

In vitro, AKR1B10 displays high catalytic efficiency for the reduction of all-*trans*-retinal, 9-*cis*-retinal, and 13-*cis*-retinal to the corresponding retinols. This catalytic efficiency exceeds that observed for short-chain dehydrogenases/reductases and medium-chain dehydrogenases/reductases implicated in the same reactions (4). The conversion of retinals to retinols can deprive retinoic acid receptors of their ligands (Fig. 1). The formation of retinal is an important junction point in retinoic acid signaling because the conversion of retinal to retinoic acid by retinaldehyde dehydrogenases is essentially irreversible. Diversion of retinals to retinols via AKR1B10 will prevent this irreversible step in the signaling pathway. This pre-receptor regulation of retinoic acid signaling may have phenotypic consequences. Fukomoto et al. also indicate that AKR1B10 is over-expressed in squamous metaplasia, a precancerous lesion of squamous cell carcinomas. The exciting observations reported in the present study suggest that AKR1B10 inhibits the cellular differentiation produced by retinoic acid. It may also provide an explanation as to why clinical chemoprevention trials by β-carotenes and retinoids fail (21). β-Carotene for example is absorbed in the intestine and by the action of dioxygenase is a source of all-*trans*-retinal and 13-*cis*-retinal. AKR1B10 could then reduce these retinals to retinols eliminating these signaling molecules (22).

Present Study and Future Directions

In the present study, strong evidence is provided for the over-expression of AKR1B10 in NSCLC associated with smoking. This association was established by microarray analysis and confirmed by reverse transcription-PCR in paired samples of squamous cell carcinomas and noncancerous tissue, by quantitative real-time PCR in unpaired samples, and by immunoblots and immunohistochemistry in squamous cell carcinoma. Over-expression of AKR1B10 was found to be a superior biomarker for NSCLC to keratin 5/6. A provocative hypothesis is proposed that AKR1B10 may control retinoic acid signaling and therefore impact the carcinogenesis process.

The next step would be to establish a relevant cellular model to validate: (*a*) that AKR1B10 can modulate retinal metabolism in a cellular context. This would involve studying

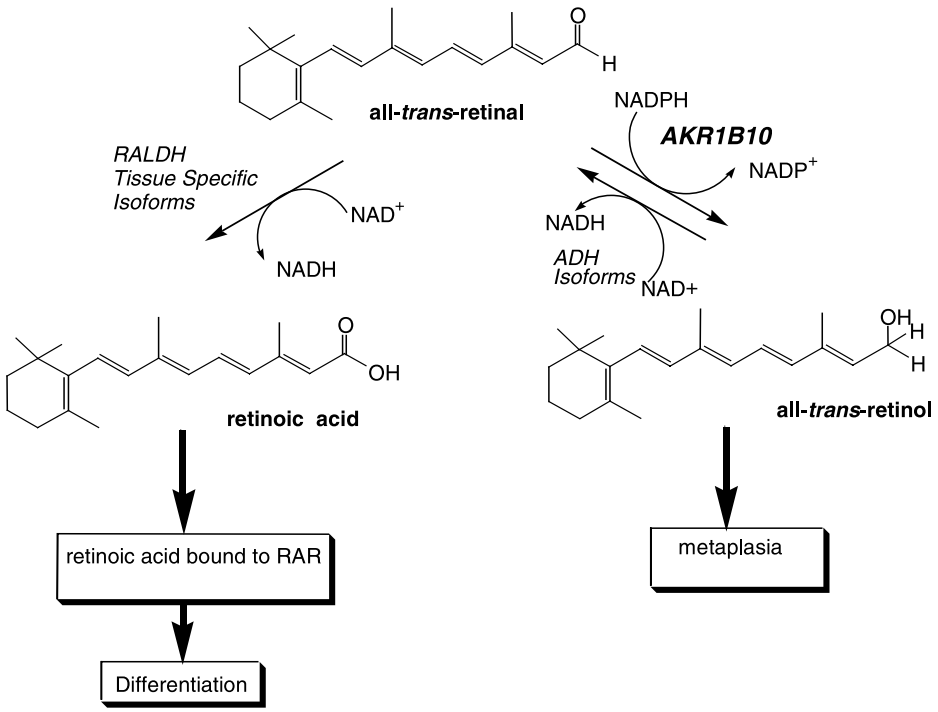

Fig. 1 Potential role of AKR1B10 in lung cell retinoic acid signaling. RALDH, retinaldehyde dehydrogenase; ADH, alcohol dehydrogenase.

retinal metabolism in AKR1B10-transfected and null cells, (*b*) that AKR1B10 can modulate retinal signaling in a cellular context. This would involve measuring changes in retinoic acid mediated *trans*-activation of retinoic acid receptors in AKR1B10 transfected and null cells, (*c*) show that AKR1B10 is colocalized with retinoic acid receptors, and (*d*) show that AKR1B10 knock-down can result in a change in retinoic acid-mediated cell differentiation. If these experiments work, it provides the possibility that AKR1B10 inhibitors could be used to enhance the chemopreventive effects of retinoids. AKR1B1 inhibitors such as tolrestat inhibit AKR1B10 with a K_i = 0.09 μmol/L (4).

However, a word of caution may be necessary. We still need to learn more about retinoid biology in lung cancer. A recent study showed that decreased expression of retinoic acid receptors and retinoid X receptors was a frequent event in NSCLC; thus, a coordinated loss of retinoic acid signaling could be occurring in this disease (23). Moreover, retinoic acid receptor-β may be proapoptotic and an inhibitor of carcinogenesis in the former smoker but it may act as an inhibitor of apoptosis and enhancer of carcinogenesis in individuals that continue to smoke (21). Nonetheless, the observations reported in the present study will not only drive improvements in early diagnosis but provide an impetus to understand more about the roles of AKR1B10 and retinoids in lung cancer progression.

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