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AKR1C3 (type 5 17β-hydroxysteroid dehydrogenase/ prostaglandin F synthase): Roles in malignancy and endocrine disorders

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Abstract

Aldo-Keto-Reductase 1C3 (type 5 17β-hydroxysteroid dehydrogenase (HSD)/prostaglandin (PG) $F_{2\alpha}$ synthase) is the only 17β-HSD that is not a short-chain dehydrogenase/reductase. By acting as a 17-ketosteroid reductase, AKR1C3 produces potent androgens in peripheral tissues which activate the androgen receptor (AR) or act as substrates for aromatase. AKR1C3 is implicated in the production of androgens in castration-resistant prostate cancer (CRPC) and polycystic ovarian syndrome; and is implicated in the production of aromatase substrates in breast cancer. By acting as an 11-ketoprostaglandin reductase, AKR1C3 generates 11β-$PGb_{2\alpha}$ to activate the FP receptor and deprives peroxisome proliferator activator receptorγ of its putative PGJ_2 ligands. These growth stimulatory signals implicate AKR1C3 in non-hormonal dependent malignancies e.g. acute myeloid leukemia (AML). AKR1C3 moonlights by acting as a co-activator of the AR and stabilizes ubiquitin ligases. AKR1C3 inhibitors have been used clinically for CRPC and AML and can be used to probe its pluripotency.

Keywords

Ketosteroids; Prostaglandins; Hormone dependent malignancy; Acute myeloid leukemia; Polycystic ovarian syndrome

1. Introduction

17β-Hydroxysteroid dehydrogenase (17β-HSD) type 5 is the only human 17β-HSD that is a member of the aldo-keto reductase (AKR) superfamily (Jin and Penning, 2007), whereas, the remainder are members of the short-chain dehydrogenase/reductase (SDR) superfamily (Bray et al., 2009). 17β-HSD type 5 is also known as type 2 3α-hydroxysteroid dehydrogenase, prostaglandin (PG) $F_{2\alpha}$ synthase, and dihydrodiol dehydrogenase X (Burczynski et al., 1998). It is one of four human AKRs of the 1C subfamily that act as HSDs and is formally known as AKR1C3.

AKR1C3 is pluripotent it that it has different ratios of 3-keto-, 17-keto- and 20-ketosteroid reductase activities. Through these activities it can regulate the amounts of androgens, estrogens and progestins available for the androgen receptor (AR), estrogen receptor (ER) and progesterone receptor (PR) (Penning et al., 2000). Its 17-ketosteroid reductase activity

distinguishes the enzyme as a peripheral tissue 17β-HSD responsible for making testosterone in an intracrine manner and is distinct from the androgenic 17β-HSD (type 3 17βhydroxysteroid dehydrogenase) (Andersson et al., 1995) and the estrogenic 17β-HSD (type 1 17β-hydroxysteroid dehydrogenase) (Jin and Lin, 1999; Marchais-Oberwinkler et al., 2011).

AKR1C3 also functions as a prostaglandin (PG) $F_{2\alpha}$ synthase and converts PGH_2 to $PGF_{2\alpha}$ and converts PGD_2 to 11β-$PGF_{2\alpha}$ (Yamada et al., 2004; Suzuki-Yamamoto et al., 1999). The $PGF_{2\alpha}$ epimers act as ligands for the FP receptor to activate cell-proliferation pathways. By so doing AKR1C3 may also prevent the non-enzymatic conversion of PGD_2 to 15-deoxy-$\Delta^{12,14}$-PGJ_2,- a putative ligand for peroxisome proliferator activated receptorγ (PPARγ) which promotes cell differentiation (Desmond et al., 2003). Cross talk may also exist through these nuclear receptor signaling pathways. 15-deoxy-$\Delta^{12,14}PGJ_2$ is a reactive Michael acceptor which covalently modifies the AR which leads to its summoylation and proteasomal degradation (Kaikkonen et al., 2013). 15-deoxy-$\Delta^{12,14}PGJ_2$ also covalently modifies the DNA binding domain of ERα thereby inhibiting its transcriptional activation (Kim et al., 2007).

These diverse actions of AKR1C3 have led to lead discovery programs to identify small molecule inhibitors as chemical probes and as potential therapeutics for hormone dependent and independent malignancies of the breast, prostate, endometrium and acute myeloid leukemia (AML) as well as other endocrine disorders (e.g. polycystic ovarian syndrome) (Penning, 2017).

AKR1C3 also has several moonlighting functions that are independent of its enzyme activity and include: co-activation of the AR (Yepuru et al., 2013), regulation of the ubiquitin ligase Siah2 (Fan et al., 2015) and regulation of histone deacetylases and chromatin remodeling (Doig et al., 2016).

Several reviews of AKR1C3 inhibitors have been published including a recent patent review of compounds undergoing preclinical development for the indications mentioned (Penning, 2017). This review is thus focused on the roles of AKR1C3 in malignancies and other endocrine disorders.

2. Discovery and properties

AKR1C3 was originally cloned and expressed from a human prostate cDNA library (Lin et al., 1997). AKR1C3 consists of 323 amino acids and its predicted monomeric molecular weight is 36,853 Da. Like other AKR enzymes it is a soluble monomeric NAD(P)(H) dependent oxidor-eductase that will convert carbonyl groups (in this case ketosteroids and ketoprostaglandins) to secondary alcohols (Jin and Penning, 2007). AKR1C3 has 86% sequence identity with AKR1C1, AKR1C2 and AKR1C4 (Burczynski et al., 1998).

All AKR enzymes including AKR1C3 catalyze a sequential ordered bi bi reaction in which NADPH binds first, followed by the ketosteroid to form the central complex E•NADPH•Ketosteroid which then undergoes chemistry to yield the E•$NADP^+$ Hydroxysteroid product complex. The hydroxysteroid product then leaves followed by

$NADP^+$ to regenerate free enzyme. The NADP(H) cofactors are bound very tightly. Based on extensive mechanistic work on AKR1C2 it is predicted that for many substrates the rate of release of $NADP^+$ places an upper limit on k_{cat} (Jin and Penning, 2006; Cooper et al., 2007).

3. Protein structure-function

Forty-three crystal structures exist for AKR1C3 which reflects the intense interest of this enzyme as a drug target. Each of these enzymes display the $(\alpha/\beta)_8$-barrel that is the signature motif of the AKR superfamily. This barrel consists of an alternating arrangement of α-helix and β-strand that repeats itself eight times, where the β-strands coalesce in the middle of the structure to form a β-barrel, Fig. 1.

The catalytic tetrad Tyr55, Asp50, His117, Lys84 resides at the base of the barrel, where Tyr55 acts as the general acid-base (Schlegel et al., 1998). In AKR1C3, the C17 ketone group of a ketosteroid is expected to hydrogen bond with Tyr55 in the center of the structure as observed with 4-androstene-3,17-dione. However, in the crystal structure with testosterone two binding poses are observed and the favored pose places the C3-ketone of testosterone at the active site (Qiu et al., 2004). By analogy with other AKR1C enzymes, the carboxamide of the nicotinamide head group is bound by Asn167, Ser166 and Gln190 and the nicotinamide ring is pi-stacked against Tyr216 so that as a net result the nicotinamide ring is bound in an extended *anti*-confirmation. This orientation permits 4-pro-*R*-hydride transfer from the nicotinamide ring to the recipient C17 ketone group of the steroid. The preference for the NADPH cofactor is achieved by the presence of R276 which forms an anchor for the phosphate on 2′AMP (Jez et al., 1997).

Consistent with these features the crystal structure of the AKR1C3•$NADP^+$•PGD_2 complex shows that the carbonyl O(11) of PGD_2 forms hydrogen bonds with Tyr55 and His117 to facilitate ketone reduction, Fig. 1 (Komoto et al., 2006).

Many AKR1C3• $NADP^+$ •Inhibitor ternary complex structures have been solved. To accommodate the binding of various ligands the enzyme uses different sub-pockets (SP1, SP2 and SP3) (Byrns et al., 2011). Because the use of these subpockets is ligand dependent predicting the binding poses of new inhibitors is challenging.

4. Steroid biosynthesis

Using a combination of continuous spectrometric and fluorometric assays and discontinuous radiochromatographic assays, AKR1C1, AKR1C2 and AKR1C3 were found to display different degrees of 3-keto-, 17-keto-20-ketosteroid reductase activity with NAD(P)H as cofactor. Examination of these data showed that AKR1C1 preferentially functions as a 20-ketosteroid reductase inactivating progesterone; AKR1C2 preferentially functions as a 3-ketosteroid reductase inactivating dihydrotestosterone (DHT) and AKR1C3 preferentially functions as a 17-ketosteroid reductase converting: 4-androstene-3,17-dione to testosterone; 5α-androstane-3,17-dione to DHT; and estrone to 17β-estradiol, Fig. 2, Table 1, (Penning et al., 2000; Byrns et al., 2008, 2010; Sharma et al., 2006).

Oxidation reactions can be monitored in the reverse direction using $NAD(P)^+$ as cofactor, however, AKR1C enzymes work predominately as reductases in the cellular environment due to the following constraints: (i) the K_{eq} of the reactions favors reduction; (ii) the affinity for the NADP(H) cofactors is in the mid-nanomolar range while the affinity for the NAD(H) cofactors is in the 200 μM range; (iv) low micromolar concentrations of NADPH can inhibit the reverse NAD^+ dependent oxidation reactions (Rizner et al., 2003); and (v) in transfection experiments in mammalian cells, where AKR1C3 must use the prevailing NAD(P)(H) concentrations, only the ketosteroid reduction reactions were evident. LNCaP-AKR1C3 cells genetically engineered to mimic AKR1C3 overexpression in prostate cancer convert 4-androstene-3,17-dione to testosterone (Byrns et al., 2012) and MCF7-AKR1C3 cells genetically engineered to mimic AKR1C3 overexpression in breast cancer convert estrone to 17β-estradiol (Byrns et al., 2010). For each of the ketosteroid reduction reactions k_{cat} values are of the order of 0.2–10 min^{-1} and K_m values are in the low micromolar range yielding k_{cat}/K_m values of 6–370 min^{-1} mM^{-1}.

5. Prostaglandin biosynthesis

AKR1C3 exhibits robust $PGF_{2\alpha}$ synthase activity. It reduces PGH_2 to $PGF_{2\alpha}$ and PGD_2 to 11β-$PGF_{2\alpha}$ (Suzuki-Yamamoto et al., 1999; Matsuura et al., 1998). Examination of the k_{cat} and K_m values for the conversion of PGD_2 to 11β-$PGF_{2\alpha}$ shows that this reaction is highly favored over ketosteroid reduction, yielding k_{cat}/K_m values of 1270 min^{-1} mM^{-1}, Table 1 (Matsuura et al., 1998) and Fig. 3.

AKR1B1 (aldose reductase) also displays robust conversion of PGH_2 to $PGF_{2\alpha}$ suggesting that this enzyme may substitute for AKR1C3 as a PGF synthase using PGH_2 as substrate (Lacroix Pépin et al., 2014). In the absence of 11-ketoprostaglandin reductase activity, PGD_2 undergoes a series of dehydration and isomerization steps to yield 15-deoxy-$\Delta^{12,14}$-PGJ_2. 15-deoxy-$\Delta 1^{2,14}$-PGJ_2 is a putative ligand for PPARγ (Kliewer et al., 1995; Reginato et al., 1998) and covalently reacts with a cysteine residue in the ligand binding domain to activate the receptor (Shiraki et al., 2004). PPARγ usually heterodimerizes with RXR (Gearing et al., 1993). AKRs also regulate the formation of RXR ligands. AKR1B10 and AKR1B15 act as the predominant all-*trans*-retinaldehyde and 9-*cis*-retinaldehyde reductases, respectively (Crosas et al., 2003; Gallego et al., 2006; Giménez-Dejoz et al., 2015). As 9-*cis*-retinoic acid is the major ligand for RXR (Heyman et al., 1992; Levin et al., 1992), the conversion of 9-*cis*-retinal to 9-*cis*-retinol mediated by AKR1B15 would deprive RXR of its 9-*cis*-retinoic acid ligand. Thus, both AKR1C3 and AKR1B15 prevent ligand access to PPARγ and RXR, respectively and inhibitors of both enzymes would inhibit cell proliferation. In another mechanism, 11β-$PGF_{2\alpha}$ binds to the Gq coupled FP receptor to activate extracellular signal-regulated kinase ½ (ERKs)-activated protein kinases (MAPKs) signaling which attenuates PPARγ transactivation by inhibitory phosphorylation leading to cell proliferation (Sales et al., 2004; Burgermeister and Seger, 2007).

6. Genetics and epigenetics

6.1. Gene structure

The *AKR1C3* gene is located on chromosome 10p15-p14 with a cytogenetic band at 10p15.1. The *AKR1C3* gene consists of 9-exons and 8 introns. No murine *akr1c* gene exists which is a functional paralog of the *AKR1C3* gene and thus the construction of *akr1c3* gene knockout animal is not feasible (Veliça et al., 2009). AKR1C6 is the closet homolog of AKR1C3 based on its activity. AKR1C6 acts as a 17β-HSD (Rheault et al., 1999) and acts as a prostaglandin F synthase due to its ability to conduct 9-ketoreduction on PGE_2 to produce $PGF_{2\alpha}$ (Veliça et al., 2009) but 9-ketoreduction of PGE_2 is not seen with AKR1C3. AKR1C6 did not reduce the 11-keto group of PGD_2 to 11β-$PGF_{2\alpha}$ which is a hallmark reaction catalyzed by AKR1C3. Thus, AKR1C6 and AKR1C3 use different PG substrates to generate distinct PGF_2 isomers.

The promoter region of the *AKR1C3* gene contains putative binding sites for the following transcription factors: Sp1, HNF-1, GATA-1, Cart-1, MRF-2 and p53 but the functionality of these transcription factors to regulate *AKR1C3* expression has yet to be demonstrated. The AKR1C3 promoter contains 15 consensus sequences for the antioxidant response element (ARE) suggesting that the enzyme is regulated by the Nrf2-Keap1 system (Tebay et al., 2015). This raises the interesting prospect that AKR1C3 is regulated by oxidative and electrophilic stress. In the prostate, *AKR1C3* is repressed by androgens binding to the AR (Powell et al., 2015) and it is upregulated by androgen deprivation therapy (ADT) in castration-resistant prostate cancer (CRPC) so that the tumor can make its own androgens as an adaptive response to castration (Stanbrough et al., 2006; Mitsiades et al., 2012; Hofland et al., 2010; Hamid et al., 2012).

Examination of the *AKR1C3* promoter shows the absence of 5′-CpG-3′ islands and thus gene expression is unlikely to be regulated by methylation status. However, transcription factors implicated in *AKR1C3* gene-regulation e.g. Nrf2-Keap1 system are regulated by methylation status (Guo et al., 2015). Thus, *AKR1C3* gene expression may be regulated epigenetically indirectly.

6.2. Splice variants

AKR1C3 transcripts can undergo alternative splicing to from two potential splice variants, P42330-1 has amino-acids 1–119 absent, and P42330-2 has only the first 204 amino acids present. Evidence that these splice variants are translated into proteins is lacking and neither are predicted to be active.

6.3. Single nucleotide polymorphisms (SNPs)

SNPs have been identified in the non-coding region of the *AKR1C3* gene and a large number of non-synonymous SNPs have been identified in its coding region. Molecular epidemiology studies have identified associations between these SNPs and disease. However, while these associations exist not all the SNPs have been shown to be of functional consequence.

Mutations in the promoter gene of *AKR1C3* C-71A > G and c-210A > C have been associated with 21-hydroxylase deficiency genotypes and may contribute to the external genital virilization observed in female's due to increased fetal androgen biosynthesis mediated by AKR1C3 (Kaupert et al., 2016). The intron variant rs1937845 which has a global minor allelic frequency (MAF) of 42% was associated with a significant increase in polycystic ovarian syndrome in Chinese women, where again this could be due to increases in androgen production (Ju et al., 2015).

Bioinformatics tools e.g. SIFT and PolyPhen2 predict that amino acids in evolutionary conserved amino acids within a protein superfamily would be deleterious to function (see Table 2) (Kumar et al., 2009; Tyler et al., 2010; Yandell et al., 2008), Functional analysis shows that H5Q, E77G, K104D, P180S, and R258C had a k_{cat}/K_m decrease of between 40 and 200 fold for the 17-ketosteroid reduction of exemestane (Platt et al., 2016). Both P180S and R258C are predicted to be loss of function mutations based on their evolutionary conservation. The occurrence of the H5Q mutation (where the C and G allelic frequencies are 53.4% and 46.6%, respectively) were associated with changes in health related quality of life seen with ADT in prostate cancer where response was superior with the minor allele (Karunasinghe et al., 2016). However, the effect of H5Q on androgen biosynthesis remains to be determined. The A215T mutation was shown to be associated with penile hypospadias likely due to diminished androgen biosynthesis (Soderhall et al., 2015).

6.4. Gene x gene interactions

The effect of some of these SNPs were further revealed when they were used in gene x gene analysis. For example the rs12529 in *AKR1C3* in association with AR-CAG repeat length was associated with a 13.7-fold increase in prostate-cancer specific mortality (Yu et al., 2013). Based on the presumed loss of 17-ketosteroid activity associated with this variant this result is unexpected. One explanation is that the effect of the allele on enzyme activity may be substrate dependent. AKR1C3 is known to exhibit alternative binding modes for different steroidal and nonsteroidal substrates and inhibitors (Byrns et al., 2011; Penning et al., 2015).

6.5. Gene and protein expression

AKR1C3 gene expression is observed in many tissues. Measurements of expression have used Northern analysis, semi-quantitative-RT-PCR and real-time qPCR for transcript analysis and immunoblot analysis and immunohistochemistry for protein expression. Immunochemical methods often use a monoclonal AKR1C3-Ab developed in our laboratory which does not cross react with any other human AKRs (Lin et al., 2004). Early Northern blot analysis demonstrated high AKR1C3 expression in liver, lung, small intestine, prostate and mammary gland (Penning et al., 2000). Others have since shown expression of AKR1C3 in the adrenal gland (Nakamura et al., 2009), brain, kidney, liver, lung, mammary gland, placenta, small intestine, colon, spleen, prostate and testis. In the prostate, immunohistochemistry has shown higher levels of AKR1C3 in epithelial cells than in stromal cells and even higher expression in adenocarcinoma of the prostate (Fung et al., 2006). AKR1C3 is expressed in male and female brain tissue but significantly higher mRNA expression and enzyme activity was observed in subcortical white matter than in the cerebral

cortex. The AKR1C3 activity was higher in adults than children but no gender differences were observed (Steckelbroeck et al., 2001).

7. Role in malignant disease

7.1. Breast cancer

AKR1C3 contributes to breast cancer development through both its 17β-HSD and $PGF_{2\alpha}$ synthase activities. AKR1C3 is overexpressed in some but not all breast cancer patients and its overexpression correlates to poor prognosis (Jansson et al., 2006; Suzuki et al., 2007). MC7-cells are AKR1C3 null but stable expression to yield MCF7-AKR1C3 cells to mimic its overexpression in breast cancer showed rapid conversion of progesterone to 20α-hydroxyprogesterone; reduction of 4-androstene-3,17-dione to testosterone; and reduction of estrone to 17β-estradiol. These studies suggest that AKR1C3 acts as a peripheral source of C19 steroid substrates for aromatase and this is likely important in post-menopausal women who no longer have functional ovaries (Byrns et al., 2010).

MCF-AKR1C3 cells also converted PGD_2 to 11β-$PGF_{2\alpha}$ and in so doing the formation of the 15-deoxy-$\Delta^{12,14}$-PGJ_2 was blunted, and growth inhibition mediated by the conversion of PGD_2 to 15-deoxy-$\Delta^{12,14}$-pGJ_2 was attenuated (Byrns et al., 2010). MCF-7 cells lack the FP receptor and thus functional consequences of 11β-$PGF_{2\alpha}$ could not be determined. MCF-7 cells stably expressing the FP receptor (MCF-FP) showed increased phosphorylation of ERK and CREB following 11β-$PGF_{2\alpha}$ treatment which resulted in an increase in Slug expression. Immunohistochemical staining showed a correlation between FP receptor and Slug expression in breast cancer (Yoda et al., 2015). Slug is a member of the SNAIL family of C2H2 zinc finger transcription factors that binds to E-box motifs. Slug is known to be a critical driver of malignant behavior including invasion, metastasis and acquisition of therapeutic drug resistance through repression of programmed cell death (Kajita et al., 2014; Iseri et al., 2011; Kim et al., 2014). Thus, the AKR1C3/FP/Slug axes may be critical in driving invasive breast cancer independent of nuclear receptors.

7.2. Prostate cancer

AKR1C3 plays a pivotal role in all pathways to androgens in prostate cancer, Fig. 4. AKR1C3 catalyzes the conversion of 4-androstene-3,17-dione to testosterone via the canonical pathway to DHT (Byrns et al., 2012; Fankhauser et al., 2014); it catalyzes the conversion of 5α-androstane-3,17-dione to DHT by the alternate pathway to DHT (Lin et al., 1997; Chang et al., 2011); it catalyzes the conversion of androsterone to 5α-androstane-3α,17β-diol, the penultimate step in the backdoor pathway to DHT (Penning et al., 2000; Auchus, 2004); and it catalyzes the conversion of DHEA to Δ^5-androstene-3β, 17β-diol an immediate precursor of testosterone. The pathway to DHT in the prostate may well be dependent on prostate cancer cell type and tissue biopsy due to the heterogeneity of the tumor. Irrespective of the pathway utilized, AKR1C3 plays a central role in all of them.

Comparison of the level of 17β-HSD expression in a panel of prostate cancer cell lines showed that *AKR1C3* transcripts were 100–1000 times higher than either *HSD17B3* or

HSD17B6, demonstrating that AKR1C3 is the major 17β-HSD in these prostate cancer cell lines, Fig. 5.

AKR1C3 is one of the most overexpressed steroidogenic genes in CRPC the fatal form of prostate cancer. In this disease AKR1C3 expression is high in both the prostate and metastatic soft tissue (Stanbrough et al., 2006; Mitsiades et al., 2012; Hofland et al., 2010; Hamid et al., 2012; Rizal et al., 2012). This overexpression is an adaptive response to low serum androgens so that the tumor can make its own androgens and it can be seen in prostate cancer cells cultured in androgen deprived media and in prostate cancer cell xenografts grown in castrate mice (Hofland et al., 2010). AKR1C3 is repressed by AR and AR agonists, but this repression can be surmounted by the expression of the fusion protein TMPRSS2-ERG which appears in late stage disease as determined by high Gleason Grade. In the proposed model, TMPRSS2-ERG displaces AR from the AKR1C3 promoter to induce AKR1C3 expression. Androgens made by AKR1C3 can further induce TMPRSS2-ERG expression providing a feed-forward model for AKR1C3 expression and enhanced intratumoral androgen biosynthesis (Powell et al., 2015).

AKR1C3 has been examined as a promising biomarker for prostate cancer progression where its high expression was measured by immunohistochemistry in 60 human prostate needle biopsies and 10 LNCaP xenografts grown in castrate male mice. Positive correlations were found between Gleason Grade and AKR1C3 expression and in the xenografts of castrated mice (Tian et al., 2014). In another study, high AKR1C3 levels were observed in a subset of CRPC patients and was found useful as a biomarker for active intratumoral steroidogenesis in biopsy or transurethral resection of prostate specimens (Hamid et al., 2012). Gene expression profiling in 20 normal prostate tissue samples, 127 primary prostate carcinomas and 19 metastatic prostate cancer specimens followed by RT-qPCR showed high expression of AKR1C3 in the metastatic prostate cancer specimens and in circulating tumor cells further validating its biomarker potential (Mitsiades et al., 2012).

The overexpression of AKR1C3 in CRPC has been modeled in an orthotopic VCaP human prostate cancer xenograft model in castrate mice where *CYP17A1, AKR1C3* and *HSD17B6* and AR splice variants were induced in response to castration (Knuuttila et al., 2014).

Two new drugs are now available clinically for the treatment of CRPC. Abiraterone acetate (a CYP17A1 inhibitor) and Enzalutamide (a AR antagonist) (Scher et al., 2010; de Bono et al., 2011; Danila et al., 2010; Attard et al., 2008). Clinical experience with these agents demonstrates that drug resistance emerges within 3–4 months. AKR1C3 overexpression contributes to abiraterone and enzalutamide drug resistance. In *in vitro* and *in vivo* xenografts, prostate cancer cells grown to be resistant to either drug could have their drug sensitivity restored either by shRNA for AKR1C3, or by the use of indomethacin (Liu et al., 2015, 2017) a selective AKR1C3 inhibitor first identified by our group (Byrns et al., 2008).

7.3. Endometrial cancer

Endometrial cancer is a disease caused by exposure to unopposed estrogens. AKR1C3 may play a role in this disease due to its peripheral formation of testosterone (an aromatase substrate); its ability to convert estrone to 17β-estradiol; and by its ability to inactive

progesterone by converting it to 20α-hydroxyprogesterone. Expression of 23 estrogen biosynthetic genes including AKR1C3 was determined in two endometrial cancer cell lines Ishikawa and HEC-1A and a control epithelial cell line HIEEC. While changes in the steroidogenic genes was noted it was concluded that the major pathway to E2 production was mediated by steroid sulfatase hydrolyzing E1-Sulfate (Hevir-Kene and Rižner, 2015). The study was expanded to 47 pairs of endometrial cancer tissue with adjacent matched control tissue. No statistical significance was noted in the expression of transcripts for *AKR1C1, AKR1C2, AKR1C3* or *SRD5A1* (Sinreih et al., 2013).

By contrast 15-deoxy-$\Delta^{12,14}$-PGJ_2 has anti-proliferative effects on endometrial cancer-derived cells (HHUA, Ishikawa, and HEC-59). Cell cycle arrest was noted at G2/M and 15-deoxy-$\Delta^{12,14}$-PGJ_2 induced AKR1C3 expression (Li and Narahara, 2013). Thus, the role of AKR1C3 in endometrial cancer may be mediated by its effects on FP and PPARγ signaling rather than by an effect on steroidogenesis and steroid receptor occupancy.

7.4. Acute myeloid leukemia (AML)

The switch between anti-proliferative signaling via PPARγ and cell-proliferation mediated by AKR1C3 production of 11β-$PGF_{2\alpha}$ has been exploited in the clinic to ameliorate acute myeloid leukemia. Thus, combination therapy of bezafibrate (a PPARγ agonist) and 6-medroxprogesterone acetate a pan-AKR1C inhibitor has been used to prevent cell proliferation (Khanim et al., 2009). However, substitution of 6-medroxyprogesterone acetate by more selective AKR1C3 inhibitors failed to show a beneficial effect suggesting that either other AKR1C enzymes play a role or 6-medroxyprogesterone acetate has other off-target effects (Khanim et al., 2014).

8. Moonlighting functions

8.1. AKR1C3 as a coactivator of AR

AKR1C3 acts as a coactivator of the AR. This function was first revealed using an AKR1C3 competitive inhibitor GTx560 (Yepuru et al., 2013) Using reporter gene assays transfection of AKR1C3 cDNA amplified the signal mediated by AR agonists. This amplified signal was attenuated with low micromolar concentrations of GTx560. Deletion mutagenesis on AKR1C3 showed that the co-activator domain of AKR1C3 was independent of the catalytic tetrad, steroid binding site or cofactor binding site. Instead the coactivator domain was located between 171 and 237 aa which comprises: α5 (170–177); β6 (188–192); α6 (200–209); β7 (212–226). Interestingly α5 contains a consensus sequence for a coactivator peptide RRQ**LEMIL** = **LXXLL** and would predict that this region could bind to the LBD of the AR. This would mean that some AKR1C3 competitive inhibitors mediate a long-range "allosteric" effect in its structure to disrupt its interaction with the AR.

8.2. AKR1C3 and Siah2 ubiquitination

Siah2 is a ubiquitin ligase that enhances AR transcriptional activity, upregulates AKR1C3 expression and enhances prostate cancer cell growth. Expression of a catalytic inactive form of AKR1C3 partially restores AR activity and cell growth in Siah2 knockdown cells (Fan et al., 2015). Unexpectedly, AKR1C3 increased Siah2 protein levels in Siah2 knockdown cells.

Siah2 binds AKR1C3 directly to inhibit its self-ubiquitination and degradation. Thus, AKR1C3 enhances Siah2 stability to increase prostate cancer cell growth via a mechanism that does not require its enzyme activity. DHX15 also stabilizes Siah2 and enhanced its E3 ubiquitin-ligase activity by binding to the nuclear export signal of AR resulting in receptor activation by stimulating ubiquitination (Jing et al., 2018). Whether AKR1C3 binds to the DHX15-Siah2 protein complex is unknown.

8.3. AKR1C3 and histone deacetylases

Histone deacetylases (HDACs) remove acetylation marks from histone proteins thereby repressing gene expression. shAKR1C3 knockdown in DuCaP and PC3 cells led to a significantly reduced mRNA expression of HDACs, NR co-activators and the PPARγ coactivator (*PPARGC1A*) (Doig et al., 2016). In addition, the histone methyl-transferase *SET7* and proto-oncogene *MYB* were significantly downregulated in shAKR1C3 treated cells. Together these results suggest that AKR1C3 is involved in chromatin remodeling independent of its roles in either AR or PPARγ signaling. It has not been determined whether the enzyme activity of AKR1C3 is required for its effect on HDAC expression.

9. Role in nonmalignant disease

9.1. Polycystic ovarian syndrome (PCOS)

PCOS is a metabolic syndrome that affects 10% of women of reproductive age. It is associated with insulin resistance and increased cardiovascular disease and androgen excess is a defining feature of the syndrome. Increased expression of AKR1C3 is observed in the disease and may be responsible for the androgen excess especially since women do not express androgenic type 3 17β-HSD. AKR1C3 mRNA was found to be higher in subcutaneous adipose tissue and was positively correlated with BMI. Insulin significantly increased AKR1C3 expression and resulted in more 4-androstene-3,17-dione being converted to testosterone in cultured subcutaneous adipose. The increased androgen production was associated with a lipotoxic lipidome in PCOS that could be reversed with an AKR1C3 inhibitor (O'Reilly et al., 2015; O'Reilly et al., 2017).

9.2. Endometriosis

Endometriosis is defined as the presence of endometrial glands and stroma outside the uterine cavity with an estimated prevalence of 6–10% in the female population and even higher in infertile women. Ectopic tissue within the pelvic cavity leads to an increase in inflammation and synthesis of FP ligands ($PGF_{2\alpha}$ and 11β-$PGF_{2\alpha}$). Higher expression of the $PGF_{2\alpha}$ synthases (AKR1B1 and AKR1C3) was observed in 22-B endometriosis cells. But only AKR1B1 was elevated in ovarian endometriosis tissue. Endometriosis is often treated with synthetic progestins (Sinreih et al., 2015). Examination of the expression of AKR1C enzymes in the Z-12 epithelial cell line, a model system of peritoneal endometriosis following treatment with the synthetic progestin dhydrogesterone showed a significant reduction in the expression of SRD5A1, AKR1C2 and AKR1C3 suggesting that progestin treatment could reduce the metabolism of endogenous progesterone and its 5α-reduced metabolites (Beranič and Lanišnik Rižner, 2013). Collectively, these studies link the

expression of AKR1C3 to the pathophysiology of the disease and response to progestin therapy in endometriosis.

10. AKR1C3 inhibitors

AKR1C3 inhibitors may be warranted for the treatment of hormone dependent malignancies (breast, prostate and endometrial cancer) and in other endocrine disorders (polycystic ovarian syndrome and endometriosis). In the sub-sets of patients with these diseases, where AKR1C3 is overexpressed, AKR1C3 inhibitors would have distinct advantages over the use of aromatase inhibitors or abiraterone which would block estrogen and androgen production in all tissues, respectively. Not surprisingly, there have been substantial efforts in academia and industry to develop potent and selective inhibitors of AKR1C3, as a recent review of the patent literature has shown, Fig. 6 (Penning, 2017). This work will not be re-reviewed, but some highlights are worth mentioning.

The issue of developing inhibitors that are selective for AKR1C3 that do not inhibit other human AKRs has been solved using a variety of non-steroidal and steroidal scaffolds. As in any lead discovery program the issue is to move these compounds through preclinical evaluation and into man. The most promising advances have been in the areas of CRPC and AML.

In CRPC, the drugs of most interest would be indomethacin and indomethacin analogs that do not inhibit COX-1 and COX-2 (Liedtke et al., 2013), since *in vivo* proof of principle studies show that indomethacin can surmount drug resistance to abiraterone or enzalutamide in xenograft models (Liu et al., 2015, 2017). Such studies suggest that patients that progress on either abiraterone or enzalutamide might benefit from indomethacin provided that their AKR1C3 expression is elevated in circulating tumor cells. ASP9521 a competitive AKR1C3 inhibitor developed by Astellas showed favorable preclinical properties in terms of DMPK, and *in vitro* and *in vivo* effects on prostate cancer cell growth (Kikuchi et al., 2014). ASP9521 was taken into the first phase I/Ib clinical trial of an AKR1C3 inhibitor for CRPC (Loriot et al., 2014). In this trial, the drug was found to be well tolerated but without efficacy. However, the authors of that study indicate that the trial design may have been flawed since patients were excluded if they had recently received ADT (Loriot et al., 2014). Where ADT is known to result in upregulation of AKR1C3[47,49, 50]. Thus, the very patients that may have benefited from ASP9521 were excluded. Other promising compounds for CRPC include GTx-560 which not only inhibits AKR1C3 with nanomolar potency but also blocks its AR coactivator function (Yepuru et al., 2013); and BMT4-158 which has nanomolar potency to inhibit AKR1C3 but also acts as an AR antagonist in the low micromolar range (Chen et al., 2012). In both cases these leads need to be taken through further optimization and preclinical development.

In AML, the combination of bezafibrate and 6-medroxyprogesterone has shown clinical benefit and is becoming standard treatment of care (Khanim et al., 2009). As previously noted, 6-medroxyprogesterone is a pan-AKR1C inhibitor and when selective AKR1C3 inhibitors were substituted the combined effects on PPARγ and FP signaling were lost (Khanim et al., 2014).

In endometriosis, Bayer have developed a series of steroid based inhibitors which are potent and selective for AKR1C3 and are in preclinical development (see patents WO201345407, WO2014009274, WO2014128108, and WO02016037956).

11. Conclusions

AKR1C3 is overexpressed in a wide variety of tumors raising the issue as to whether it promotes cell proliferation or whether it is merely a biomarker of malignancy. Evidence suggests that is a major peripheral tissue 17β-HSD that can form potent androgens under castrate conditions and the same is likely to be true in post-menopausal women where the testosterone formed is a source of estrogens following aromatization by CYP19. The $PGF_{2\alpha}$ synthase activity also provides a route to ligands for the FP receptor that would be pro-proliferative. Whether AKR1C3 prevents conversion of PGD_2 to 15-deoxy-$\Delta^{12,14}$-PGJ_2 is controversial since this compound is a reactive Michael acceptor and can be scavenged by GSH (Bell-Parikh et al., 2003). However, reduced PPARγ signaling leading to a pro-proliferative response can occur via activation of the FP receptor by 11β-$PGF_{2\alpha}$ formed by AKR1C3.

The overexpression of AKR1C3 in tumors and in other endocrine disorders suggest that it is a target for drug therapy of these conditions. Clinical studies with AKR1C3 inhibitors e.g. ASP9521 and 6-medroxyprogesterone have yielded mixed success in CRPC and AML. Reasons for the lack of efficacy is that the wrong patient group was selected for CRPC or in the case of AML, the effects seen with pan-AKR1C inhibitor 6-medroxyprogesterone could not be recapitulated with more specific AKR1C3 inhibitors suggesting other AKR1C involvement. Thus, much needs to be done in these therapeutic areas.

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Abbreviations used:

ADT	androgen deprivation therapy
AML	acute myeloid leukemia
AR	androgen receptor
CPRC	castration-resistant prostate cancer
ER	estrogen receptor
PCOS	polycystic ovarian syndrome
PPARγ	peroxisome proliferator activated receptor
PR	progesterone receptor
FP	prostaglandin FP receptor

RAR	retinoic acid receptor
RXR	retinoic acid X receptor
SNP	single nucleotide polymorphism

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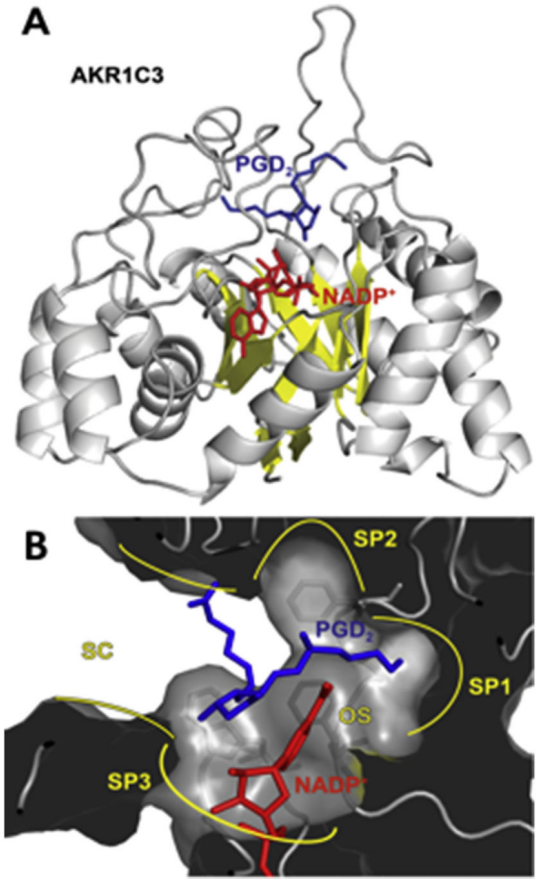

Fig. 1.

Crystal structure of the AKRC3•$NADP^+$•PGD_2 complex. (A) Side view $(\alpha/\beta)_8$ barrel (barrel in yellow) showing the perpendicular arrangement of $NADP^+$ (red) to the substrate PGD_2 (blue); and (B) proximity of the nicotinamide head group of $NADP^+$ (red) with the 11-keto group in PGD2 (blue) with the positions of the side chains of PGD_2 shown. OS = oxyanion site; SC = steroid cavity; SP1-SP2, subpockets (SP) 1–3 taken from PDB1RYO. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

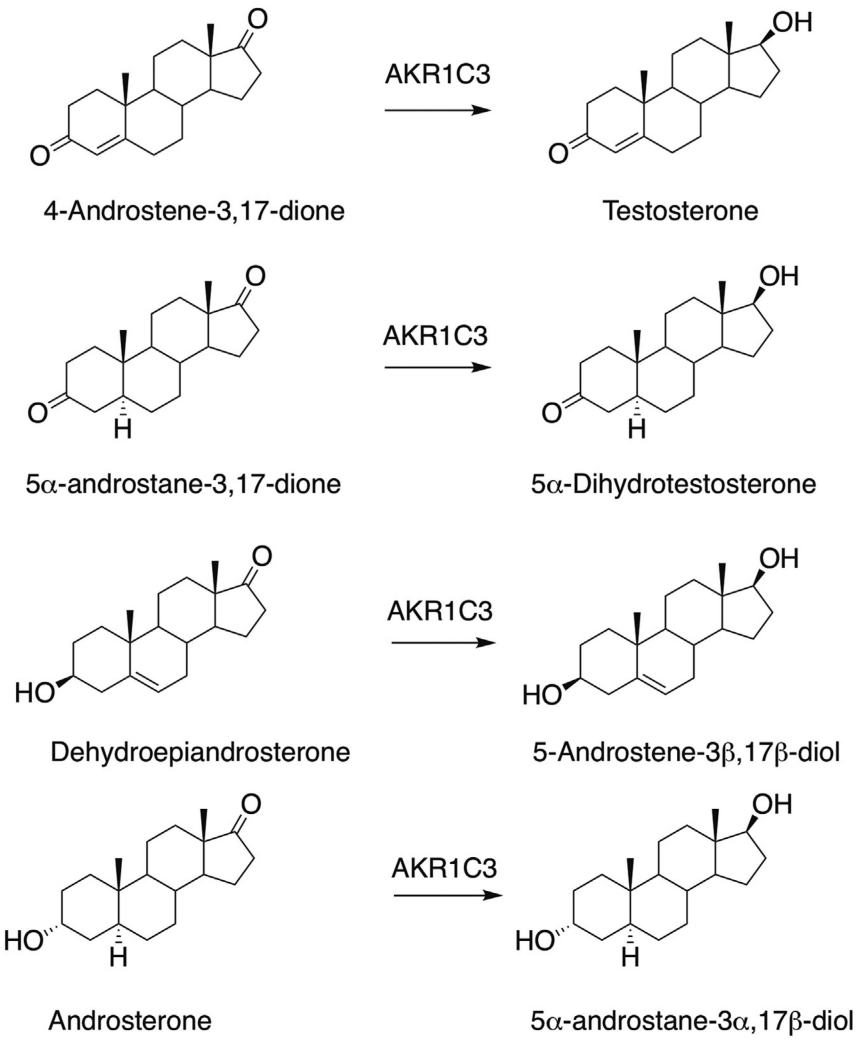

Fig. 2.

AKR1C3 as a 17β-Hydroxysteroid Dehydrogenase. Reduction of 17-ketosteroid androgens by AKR1C3.

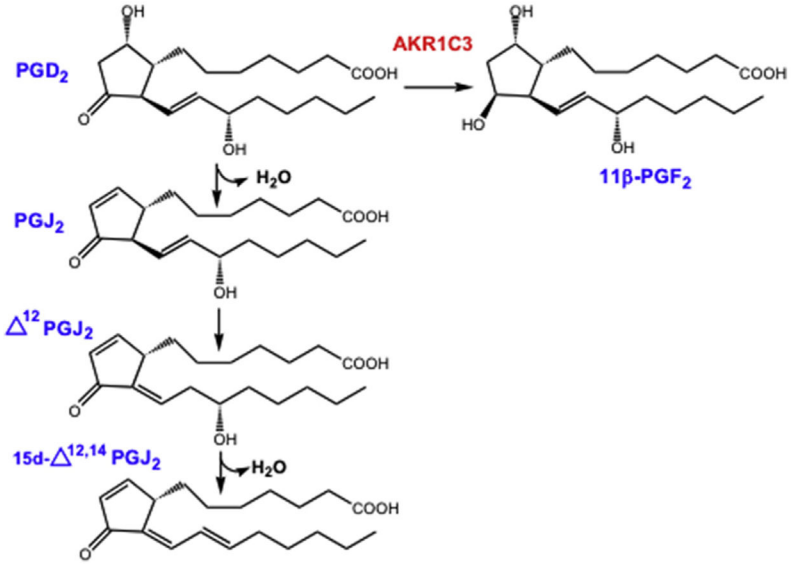

Fig. 3.

Prostaglandin $F_{2\alpha}$ synthase activity of AKR1C3. In the absence of the enzyme PGD_2 undergoes successive dehydration and isomerization steps to produce 15-deoxy-$\Delta^{12,14}$-PGJ_2.

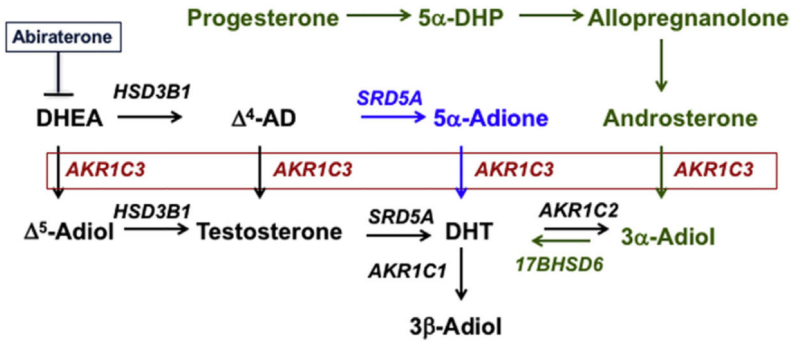

Fig. 4.

Central role of AKR1C3 in androgen biosynthesis in prostate cancer. DHEA, dehydroepiandrosterone; Δ^5-Adiol, Δ^5-androstene-3β 17β-diol; DHT, dihydrotestosterone; Δ^4-AD, 4-androstene-3,17-dione; 5α-Adione, 5α-androstane-3,17-dione; 3α-Adiol, 5α-androstanc-3α,17β-diol; 3β-diol, 5α-androstane-3β,17β-diol; 5α-DHP, 5α-dihydroprogesterone; HSD3B1, 3β-hydoxysteroid dehydrogenase type 1; SRD5A, steroid 5α-reductase; 17BHSD6, 17β-hydroxysteroid dehydrogenase type 6.

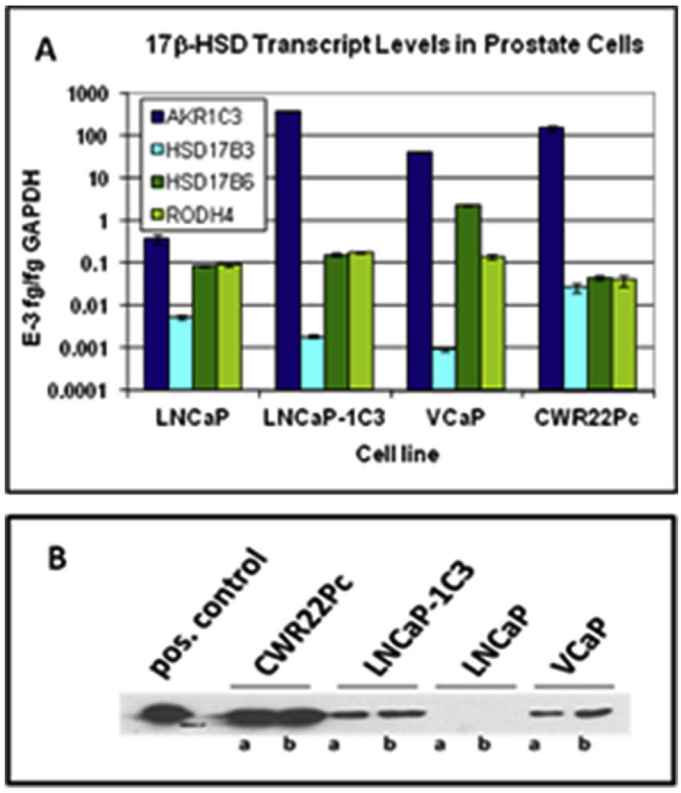

Fig. 5.

Transcript and protein expression of 17βhydroxysteroid dehydrogenase isoforms in prostate cancer cells. (A) qRT-PCR of transcripts normalized to fg GAPDH. LNCaP-AKR1C3 (LNCaP cells stably transfected with AKR1C3 cDNA): (B) immunoblot analysis of AKR1C3 protein expression in lysates from prostate cancer cells (50 μg/lane) with a positive control of recombinant AKR1C3 (0.2 μg/lane), where lanes a, b are duplicate samples (unpublished data Mindnich and Penning).

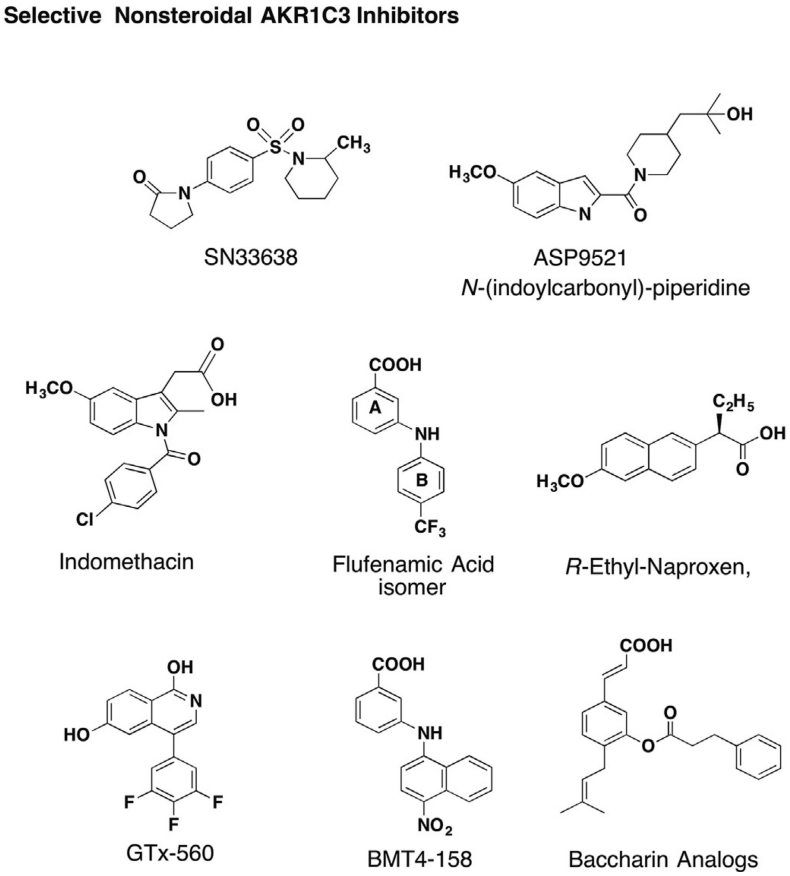

Fig. 6.
Selective nonsteroidal AKR1C3 inhibitors.

Table 1

Steady state kinetic constants for substrate turnover by AKR1C3.

Substrate	Cofactor	Reduction	K_m (μM)	*k*cat (min^{-1})	k_{cat}/K_m (min^{-1} mM^{-1})
5α-DHT (R)	NADPH	3-Ketosteroid	19.8	0.26	6
5α-androstane-3,17-dione (S)	NADPH	17-Ketosteroid	5.0	7.63	56
4-androstene-3,17-dione (R)	NADPH	17-Ketosteroid	13.4	0.87	65
Androsterone (S)	NADPH	17-Ketosteroid	8.9	10.2	42
Estrone (R)	NADPH	17-Ketosteroid	9.0	0.068	7.5
Progesterone (R)	NADPH	20-Ketosteroid	2.8	1.0	370
PGH_2	NADPH	Prostaglandin endoperoxide	10	3.7	370
PGD_2 (S)	NADPH	11-Keto-prostaglandin	1.1	1.4	1270

R = radiochemical assay

S = spectrometric assay.

Table 2

Non-synonymous single nucleotide polymorphisms in *AKR1C3*[a]

Amino Acid Position	Amino acid change	Nucleotide change	Nomenclature	MAF
5	H →Q[b]	C/G	rsl2529	0.4203
36	E→Nonsense	G/T	rsl804062	0.2410[c]
42	I→V	A/G	rs575984704	0.0020
47	R→H	G/A	rs546552035	0.0020
49	I→T	T/C	rsl45911457	0.0020
59	E→K	G/A	rsl45075384	0.0040[c]
66	R→Q	G/A	rs35961894	0.0230
76	R→G	A/G	rs370884375	0.0040[c]
77	E→G[b]	A/G	rsll551177	0.0370[c]
91	R→Nonsense	C/T	rsll8150330	0.0040
97	P→A	C/G	rs202144998	0.0020
104	K→D[b]	A/T	rsl2387	0.1518[c]
122	L→V	C/G	rs531233762	0.0010
145	C→Y	G/A	rs28943579	0.0050
163	I→T	T/C	rs200514658	0.0040
170	R→C	C/T	rs35575889	ND
175	M→I	G/C	rsll3132	ND
180	P→S[b]	C/T	rs34186955	0.0860[c]
183	K→R	A/G	rs61730879	0.0260[c]
190	Q→Nonsense	C/T	rsl40580498	0.0240
199	R→W	C/T	rsl99934766	0.0040[c]
199	R→Q	G/A	rsl39146411	0.0040[c]
208	S→L	C/T	rsll6351638	0.0010
250	R→Q	G/A	rs562560936	0.0020
258	R→C[b]	C/T	rs62621365	0.0325[c]
315	P→T	C/A	rs202095354	0.0020[c]

[a]Only SNPs with a MAF > 0.001 (0.1% are shown from the NCBI database).

[b]Change in steady state kinetic properties reported.

[c]Evolutionary conserved amino acids.