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Function of nuclear sex hormone receptors in gene regulation

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Abstract The development of reproductive organ tumors such as breast and prostate cancer often depends on the action of sex hormones. Nuclear sex hormone receptors are members of the nuclear hormone receptor superfamily and act as ligand-inducible transcription factors, controlling the expression of target genes. Nuclear receptors are considered to directly and indirectly interact with a number of nuclear co-regulatory complexes involved in chromatin remodeling and histone modification. Moreover, many intracellular signalings via cell membrane receptors are shown to modulate nuclear receptor-regulated transcription. We have shown that estrogen receptors (ER) associate with a number of nuclear complexes, one of which is a spliceosome complex. We recently found that this spliceosome complex interacts with phosphorylated ER by MAP kinase, generating a novel cross-talk of estrogen and growth factor signalings. We also observed that a dioxin receptor (AhR) is capable of associating with ER, resulting in modulation of ER transactivation function. From our findings we believe that development of estrogen-dependent breast cancer may be mediated through the other signaling pathways. To address the function of the androgen receptor (AR) in androgen-dependent prostate cancer, we established a transgenic mouse line expressing a human AR mutant that is found in androgen-independent prostate cancer patients. The hAR mutant mice, generated through a Cre-loxP system, developed hyperplasia in the prostates. Hypersensitivity of AR mutants to antagonists and endogenous steroid hormones may potentiate hormone-dependency in prostate cancer development.

Keywords Androgen receptor · Androgen receptor knockout mouse · Cre-loxP system · Testicular feminization mutation

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Function of the nuclear receptor in gene regulation

Sex steroids exhibit a wide variety of biological actions in physiological and pathological events [16, 27]. Development of reproductive organ tumors such as breast and prostate cancer often depends on the action of sex hormones, but the molecular basis remains totally unknown. Nuclear sex hormone receptors such as androgen receptors (AR) and estrogen receptors (ER) are members of the nuclear hormone receptor superfamily and act as ligand-inducible transcription factors. Both ER and AR form homodimers and bind specific DNA elements called hormone-responsive elements (HRE) in the target gene promoters [1, 4]. Members of the nuclear receptor (NR) gene superfamily serve as sequence-specific regulators in the promoters of their cognate target genes [13]. Reflecting the spatio-temporal expression patterns of NR in animals, a wide variety of biological events are under the control of NR-mediated transcriptional regulation [3, 15]. Structurally, NR proteins can be divided into five domains, A–E. The highly conserved C domain acts as a DNA-binding domain (DBD), which has two zinc finger motifs that recognize and stably bind to specific target DNA. The moderately-conserved ligand-binding domain (LBD) is mapped to the C-terminal of the E domain. The N-terminal A/B domain exhibits little homology among NRs and is responsible for ligand-induced transactivation together with the LBD region in NRs [13] as interacting regions

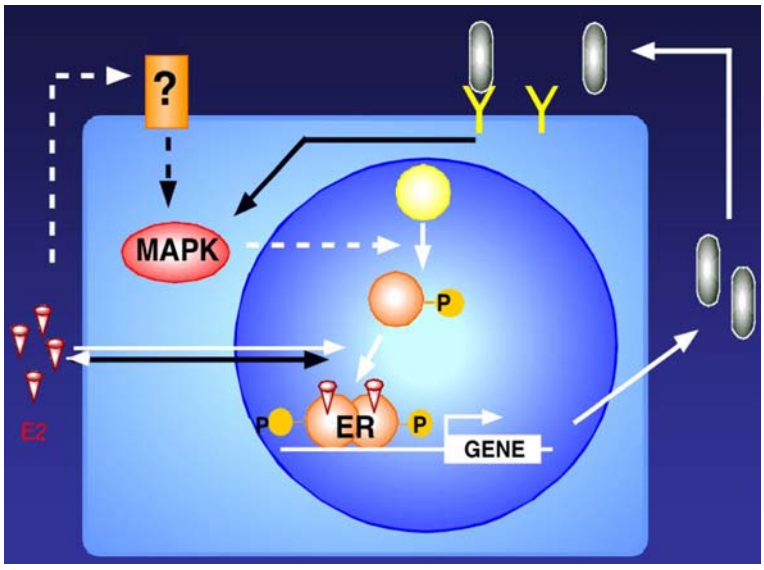

Fig. 1 MAP kinase-mediated growth factor signaling potentiates human estrogen receptor (ER)α transactivation function through Ser^{118} phosphorylation

for co-activator complexes [3, 15, 28]. The autonomous activation function (AF)-1 located in the A/B domain is ligand-independent, while AF-2 in LBD is induced upon ligand binding [24]. Unliganded LBD appears to suppress the function of the A/B domain, while ligand binding to LBD is thought to evoke the function of the LBD and restore A/B domain function through, as yet undescribed, intramolecular alteration of the entire steroid receptor structure.

Both ligand-dependent and -independent transcriptional control by NRs require the input of two types of co-regulators with opposing functions, co-activators vs. co-repressors. It appears that most co-regulators exist as multi-protein complexes [3, 15]. It is thought that three distinct classes of co-activators support NR transactivation, with two of these classes, CBP/p160 and GCN5/TRPAP complexes [20, 24, 28], containing histone acetyltransferase (HAT) enzymes. The other class, DRIP/TRAP complex, is a non-HAT co-activator complex [6, 21]. The co-repressor type complexes contain histone deacetylase (HDAC) enzymes which, along with NCoR/SMRT, physically interact with NRs via CoRNR motifs and are thought to be functionally indispensable subunits in NR co-repression complexes [5, 17]. While histone modification due to HAT/HDAC activity in NR co-regulator complexes in co-operation with chromatin remodeling complexes explains, at least in part, the mechanism of NR-mediated transcriptional control [10, 18], the molecular link between NR-mediated gene regulation and cell cycle control remains elusive.

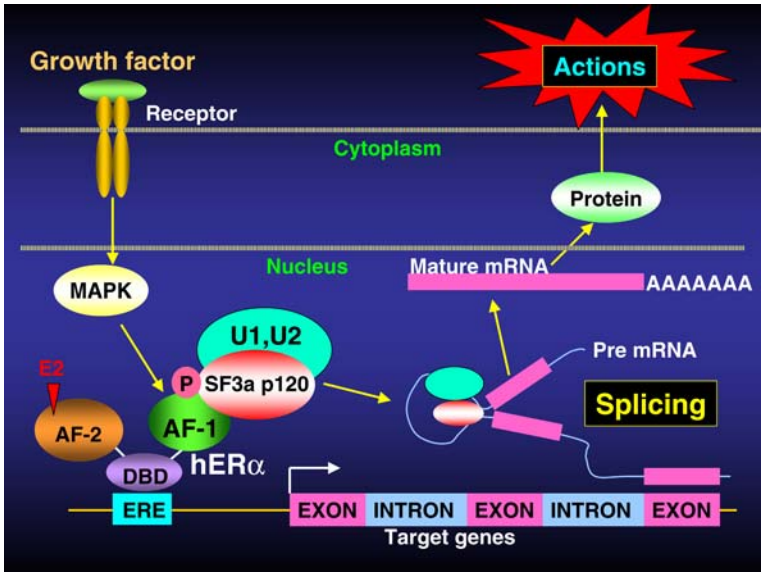

Fig. 2 MAP kinase-mediated growth factor signaling controls RNA splicing efficiency by Ser^{118} phosphorylation-dependent association with spliceosome subunit SF3a p120

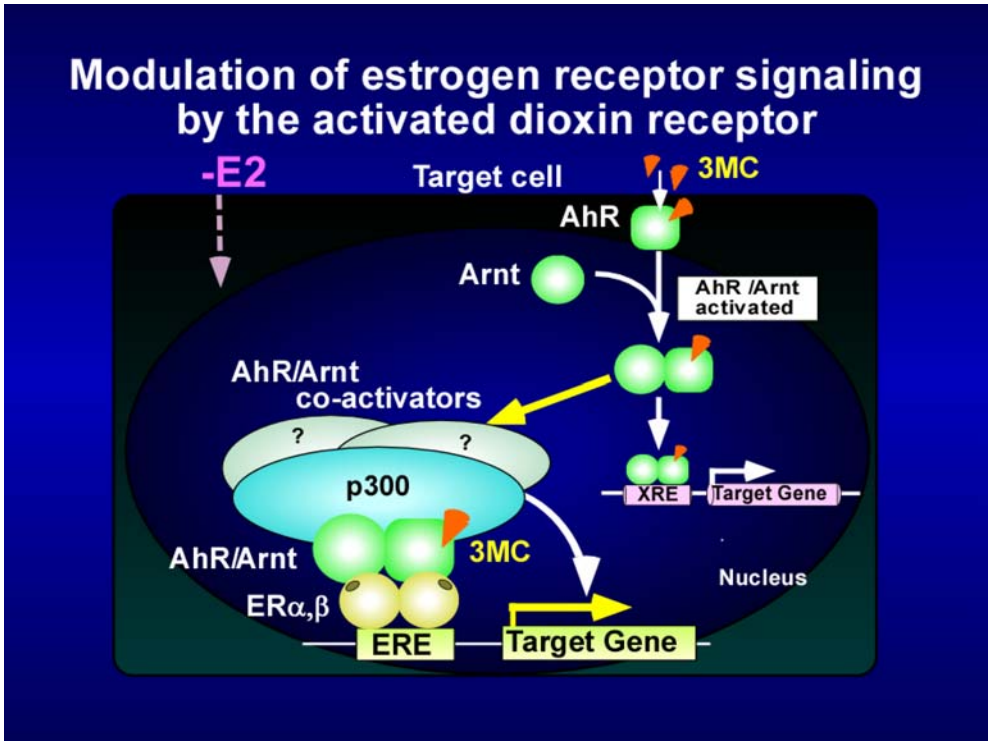

Fig. 3 Agonist-activated dioxin receptor (*AhR*)/Arnt heterodimer directly associates with estrogen receptors (*ER*)α and ERβ. This association results in recruitment of unliganded ER and co-activator p300 to estrogen-responsive gene promoters, leading to activation of transcription and estrogenic effects in the absence of estrogen

Cross-talk of ERα-mediated estrogen signaling with growth factor and dioxin signaling

MAP kinase activated by growth factor signaling potentiates ER transactivation function

It is well known that growth factors such as insulin and epidermal growth factor potentiate estrogen action in ER target tissues and estrogen-dependent breast cancer. In our previous reports, we have shown that human (h)ERα AB domain at serine (Ser)[118] is phosphorylated by MAP kinase activated by growth factor signaling, and that this phosphorylation results in the potentiation of hERα AF-1 transactivation establishing a novel cross-talk of growth factor with estrogen signaling (Fig. 1) [8]. In search of co-regulators responsible for this phosphorylation-induced transactivation of hERα

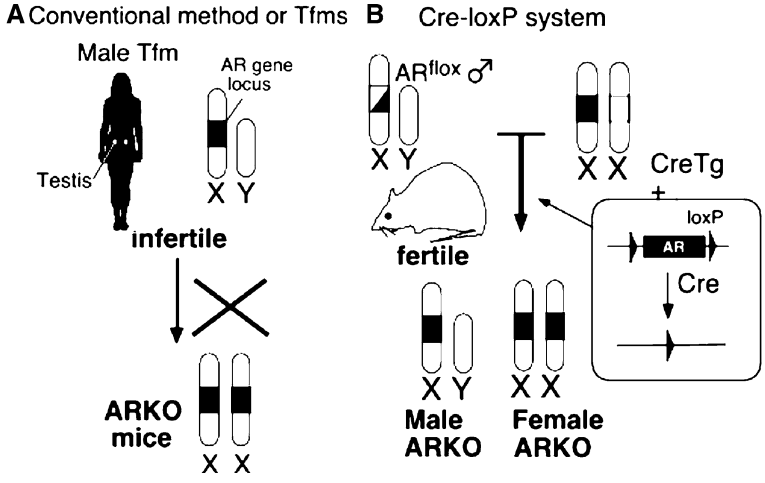

Fig. 4 Strategy for generating an androgen receptor knockout (*ARKO*) mouse line. **A** The androgen receptor (*AR*) gene is located in the X chromosome and male testicular feminization mutation (*Tfm*) animals are infertile so that the mutated AR gene cannot be transmitted to the next generation. **B** In the first step, floxed AR mice carrying functional but loxP-flanked AR gene are generated by introducing loxP sites in the first exon of the AR gene by homologous recombination in embryonic stem cells. Next, by mating these mice with CMV-Cre transgenic mice, the AR gene is disrupted during embryogenesis

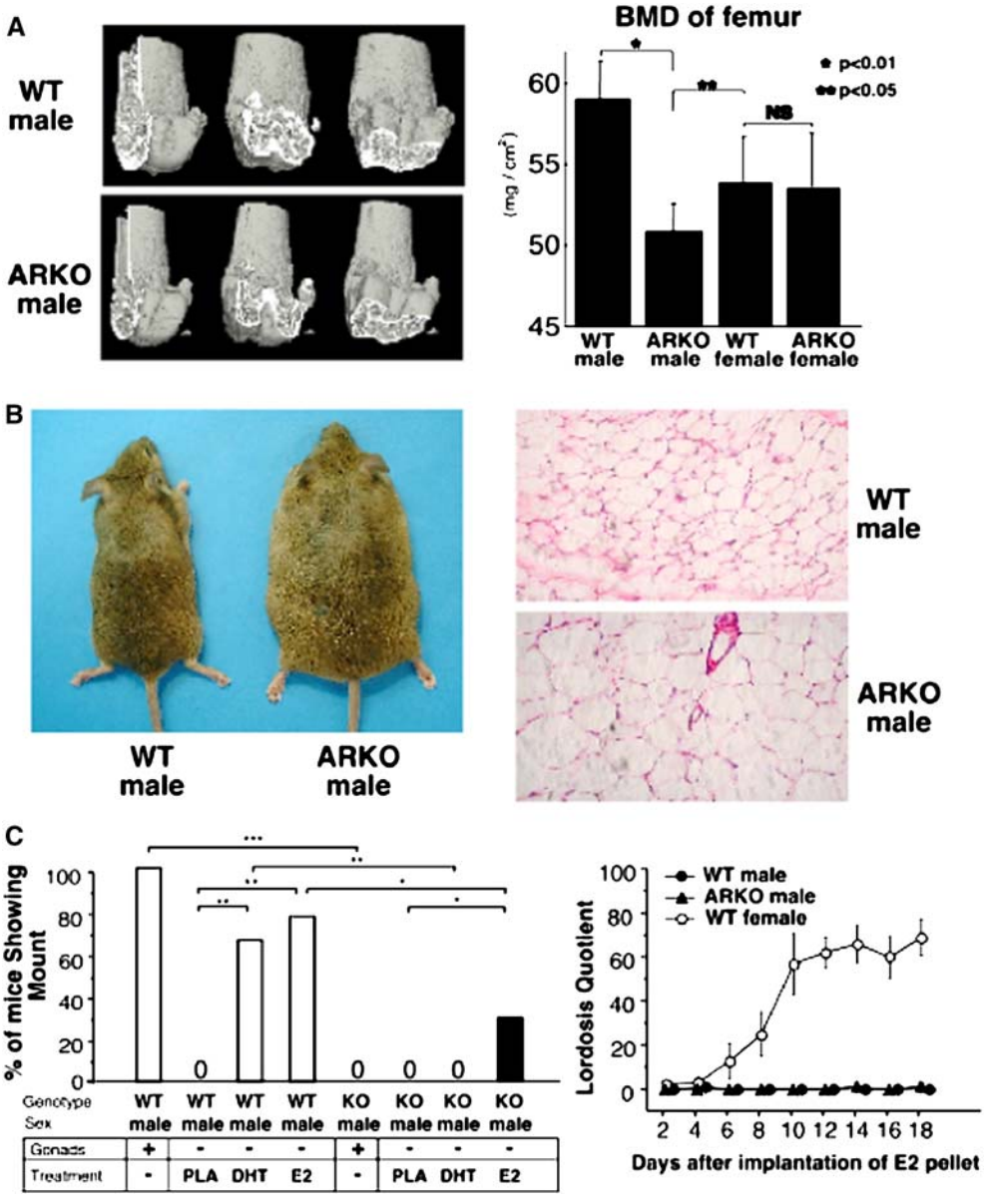

Fig. 5 Phenotypic features of androgen receptor knockout (*ARKO*) male mice. **A** Osteopenia in male ARKO mice. Three-dimensional computed tomography images of distal femora from representative 8-week-old male ARKO mice (*left panel*). Bone loss in femur of 8-week-old male ARKO mice by bone mineral density (*BMD*) analysis (*right panel*). **B** External appearance of 30-week-old male ARKO mice (*left panel*). Subcutaneous white adipose tissues from 30-week-old male ARKO mice (*right panel*). **C** Ablation of AR in male mice resulted in lack of both male and female sexual behaviors. Loss of all components of male sexual behavior in intact (Gonads: +) 10-week-old ARKO mice (*left panel*). Female sexual behavior was not induced in gonadectomized ARKO male mice after treatment with 17β-estradiol (*E2*) (*right panel*)

AF-1, we identified p68/p72 as hERα AF-1-specific co-activators, which were revealed to be components of the known ERα AF-2 HAT co-activator complex [2, 26]. However, in vitro interaction of p68/p72 with hERα AB domain was enhanced by MAP kinase-mediated phosphorylation, but not completely dependent. This observation led us to seek other co-regulators. Using Far-Western blotting, we detected a p120 factor as a phosphorylation-dependent interactant. Molecular cloning of this p120 factor revealed that p120 is a component of the known spliceosome complex. Splicing factor SF3a p120 serves as a co-activator specific for hERα AF-1. The physical association of SF3a p120 with hERα is dependent on the phosphorylation of hER Ser^{118} by MAP kinase, which is activated by either MAPKK or k-Ras^{val12}, common downstream factors of growth factor signaling. Transactivation and splicing assays revealed that SF3a p120 potentiates hERα-mediated splicing with transcriptional co-activation. Most notably, ERα-mediated potentiation of RNA splicing by SF3a p120 requires phosphorylation of hERα Ser^{118} by activated MAP kinase. Hence these findings suggest a mechanism whereby growth factor signaling regulates gene expression through the modulation of RNA splicing efficiency by phosphorylation of sequence-specific activators following association of activators with the spliceosome (Fig. 2) [14].

Modulation of ER transactivation by dioxin receptor (AhR)-mediated signaling

Environmental contaminants are known to affect a wide variety of biological events in many species. Dioxins, typical environmental contaminants, exert adverse estrogen-related effects [22]. While dioxins are well known

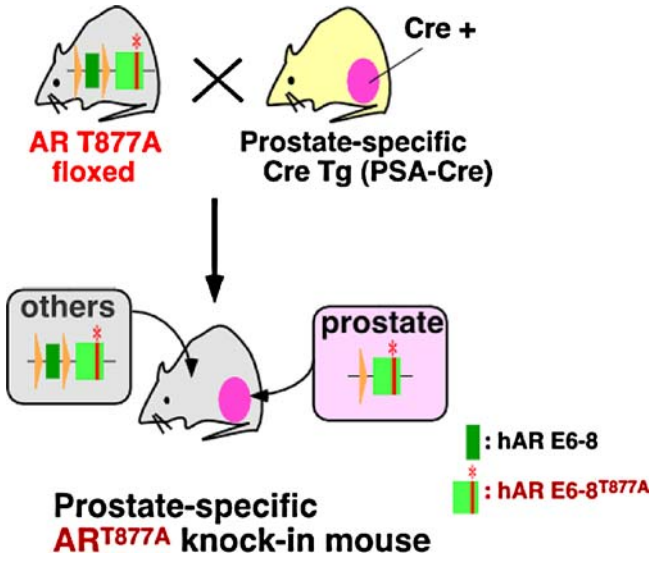

Fig. 6 Strategy for generating prostate-specific androgen receptor (*AR*) knock-in mice expressing AR^{T877A} mutation. Floxed AR mice that express human–mouse hybrid AR but loxP-flanked AR gene are generated by homologous recombination in embryonic stem cells. Next, by mating these mice with prostate-specific Cre transgenic mice, wild-type AR gene could be replaced with human mutant AR gene only in prostate

to exert antiestrogenic actions, they are also reported to induce endometriosis and estrogen-dependent tumors, implying possible estrogenic effects [25]. However, the molecular mechanism underlying such estrogen-related actions of dioxins remains largely unknown. The heterodimer of dioxin receptor (AhR) and AhR nuclear translocator protein (Arnt) is known to mediate most of the toxic effects of dioxins [11]. We demonstrate here that agonists-activated AhR/Arnt heterodimer directly associates with ERα and β. This association induces recruitment of unliganded ER with AhR/Arnt and co-activator p300 to estrogen-responsive gene promoters, activating transcription and exerting estrogenic effects, while attenuating the function of liganded ER. The estrogenic actions of AhR agonists were detected in wild-type ovariectomized mouse uteri, but were absent in AhR–/– and ERα–/– ovariectomized mice [19]. Our findings suggest a novel mechanism whereby ER-mediated estrogen signaling is modulated by a co-regulatory-like function of activated AhR/Arnt, giving rise to adverse estrogen-related actions of dioxin-type environmental contaminants (Fig. 3).

Function of hAR mutant in prostate development

To study the function of AR mutants found in the intact prostate of prostate cancer patients, a hAR mutation (T877A) was introduced into AR gene floxed mice [7, 9] by replacing the mouse AR LBD with hAR LBD.

Generation of floxed AR mice

The molecular basis of AR function, especially regarding disease states, remains largely uncertain due to the lack of stable genetic models. Basic and technical difficulties are faced with generating AR knockout (ARKO) mice (Fig. 4). The AR gene is located on the X chromosome, thereby existing as a single copy in karyotype 46 XY males, in which androgen exerts its most profound effects. Since male mice lacking the AR gene are expected to show testicular feminization mutation (Tfm) abnormalities with complete infertility [23], successful targeted disruption of the AR gene, which is essential for reproduction, necessarily prohibits its transmission to subsequent generations. It is thus impossible to generate an ARKO mouse line either in nature or by conventional gene targeting method. Furthermore, because all Tfm models are genetically male, it is impractical to generate genetically female animals homozygous for the AR gene mutation.

To overcome this problem, we applied a Cre-loxP system [12] to establish an ARKO mouse line. We first generated floxed AR mice, in which the AR gene locus was flanked by loxP sites. Floxed AR mice were fully fertile and expressed AR protein normally. We then crossed them with mice expressing Cre recombinase ubiquitously under the control of the cytomegalovirus (CMV) promoter, and obtained male and female ARKO mice at theoretical Mendellian frequency. Male ARKO mice exhibited a number of Tfm phenotypes such as female-typical external appearance, vagina with blind end and clitoral-like phallus. They also showed loss of bone mass with late-onset obesity and sexually behaved as female littermates (Fig. 5) [7, 9, 23]. Abnormal

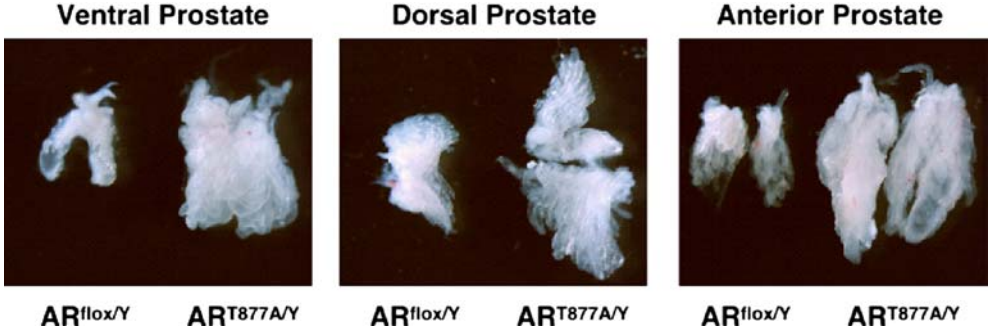

Fig. 7 Enlarged ventral, dorsal, and anterior prostate of 17-week-old AR^{T877A} mutant mice

ovarian development was seen in female ARKO mice (Shiina et al., unpublished results).

Genetic introduction of a hot-spot hAR LBD mutation of prostate cancer patients into a mouse model

The floxed mice were then applied for a knock-in approach (Fig. 6). Mice expressing mouse–human hybrid AR mutant protein (AR [T877A/Y] mice) were normal with regard to external organs and reproduction. However, prostate size in AR (T877A/Y) mice observed at age 17 weeks was clearly increased (Fig. 7). No antagonistic action of hydroxyflutamide in prostate development was observed. Thus these findings suggest that hypersensitivity of AR mutants to antagonists and endogenous steroid hormones may potentiate hormone-dependency in prostate cancer development. Currently, development of a prostate-specific knock-in is underway by means of PSA-Cre tg mice.

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