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Function of androgen receptor in gene regulations☆

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Abstract

Most of the androgen actions are considered to be mediated by the androgen receptor (AR) of the target genes. The AR is composed of a fairly large molecule because of the long A/B domains of its N-terminal. However, the independent roles of the AR as well as those of the estrogen receptors largely remained unknown mainly due to the lack of the AR knockout (ARKO) mice line. We have succeeded in generating the ARKO mouse by means of a conditional targeting using the Cre/loxP system. The ARKO males grew healthily although they showed a typical feature of the testicular feminization mutation (Tfm) and the hormonal assay revealed significantly lower serum androgen and higher LH levels in comparison with those of the wild type (WT) males. The serum estrogen levels were, however, comparable between both the ARKO and the WT. Another hallmark of the ARKO males was a state of high bone turnover osteopenia, in which the acceleration in the bone resorption clearly exceeded the bone formation. Male-typical behaviors were disrupted in male ARKO mice. Aiming at a quick differentiation of an androgen-dependent polyQ disease such as Kennedy's disease, the authors also developed the Drosophila fly-eye model in which the wild type and the polyQ-expanded human AR (hAR) was induced in the eyes of Drosophila. When androgen was administered to the flies induced with the polyQ-expanded hAR, their optical nerves were devastated.

Keywords: Androgen; Androgen receptor; KO mice; Transgenic fly

1. Introduction

The androgen receptor (AR), a member of the steroid hormone receptors superfamily, is composed of a fairly large protein in comparison with thyroid hormone receptors (TR), Vitamin D receptors (VDR), retinoid receptors (RXR) as well as estrogen receptors [1–3]. It is because the A/B domains of the N-terminal of the AR that include a polyQ repeat are much longer than those of other receptors [4–6]. Androgen controls the expression of genes via the AR, in which the AR positively or negatively regulates the expression of the target genes acting as androgen-dependent transcription factors, under the existence of co-activators [5–7]. When the AR functions on the DNA of the genes, the complex of the co-activators interact as a trigger with the basal transcription factor and the AR for initiating the transcription.

Recent studies of two subtypes of estrogen receptors, ERα and ERβ, found that, especially in the knockout mouse, a clear phenotypes such as osteoporosis were not manifested perhaps because the plasma level of androgen had been extremely elevated [8]. This may be explained by the fact that androgen is the precursor of estrogen in the female mouse. It has been also reported that in the aromatase knockout female mouse, the circulating testosterone levels are markedly elevated [9]. Such being the case, there was a demand in developing the androgen receptor knockout (ARKO) mouse to investigate the actions of sexual steroid hormones individually. Androgen is required for the genital organs as well as sexual behavior not only in males but also in females. And in the clinical aspect, it is well known that some prostatic cancer can be androgen-dependently aggravated. A clarification of these issues was also expected with the development of the ARKO mouse.

2. Phenotypes of androgen knockout mouse

There were basic and technical difficulties in generating an ARKO mouse. When the AR gene is mutated in the male mouse, the mouse turns out phenotypic female without having a normal female or male genitalia and is infertile [10,11]. Moreover, as the AR gene is located only

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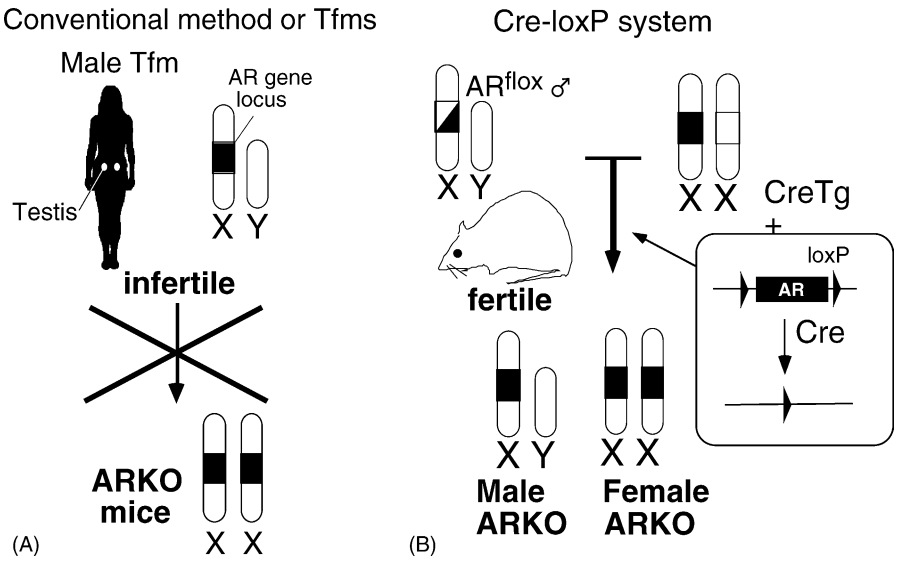

Fig. 1. Strategy for generating ARKO mice line when the male ARflox mouse with a partially modified AR gene induced by lox P and the female transgenic mouse ($CreTg^{+}$) generated by applying recombinase Cre were mated, all the AR genes were disrupted during the embryogenesis; thus, an ARKO mice line was obtained.

on the X chromosome, there is no male hetrozygoute of the AR gene-dirsrupted animals to transfer the mutated AR gene. It is thus impossible to obtain a female homozygote by either naturally occurring genetic mutaions or conventional targeted gene disruption method. Thus, the animal which has a recessive genotypic change in the AR gene can not be generated by means of the usual methods.

Such being the case, we planned to introduce the recombinase Cre/Lox-P base sequence (Cre-lox P system) into the mouse AR gene locus (Fig. 1) to generate ARKO mice line [12]. To begin with, we generated a potential AR knockout (ARKO) mouse (floxed ARf) by introducing the lox P, a capsid of a DNA breaking enzyme, in the AR gene by homologous recombination in ES cells. Three lox P sites were successively introduced in the first intron of the mouse AR gene. The male floxed AR mice are completely fertile/normal so far, and showed a normal expression and function of the AR, nevertheless, under the partially modified AR gene. On the other hand, a female transgenic mouse was generated by applying the recombinase Cre, which induces a recombination at the site between the two lox P

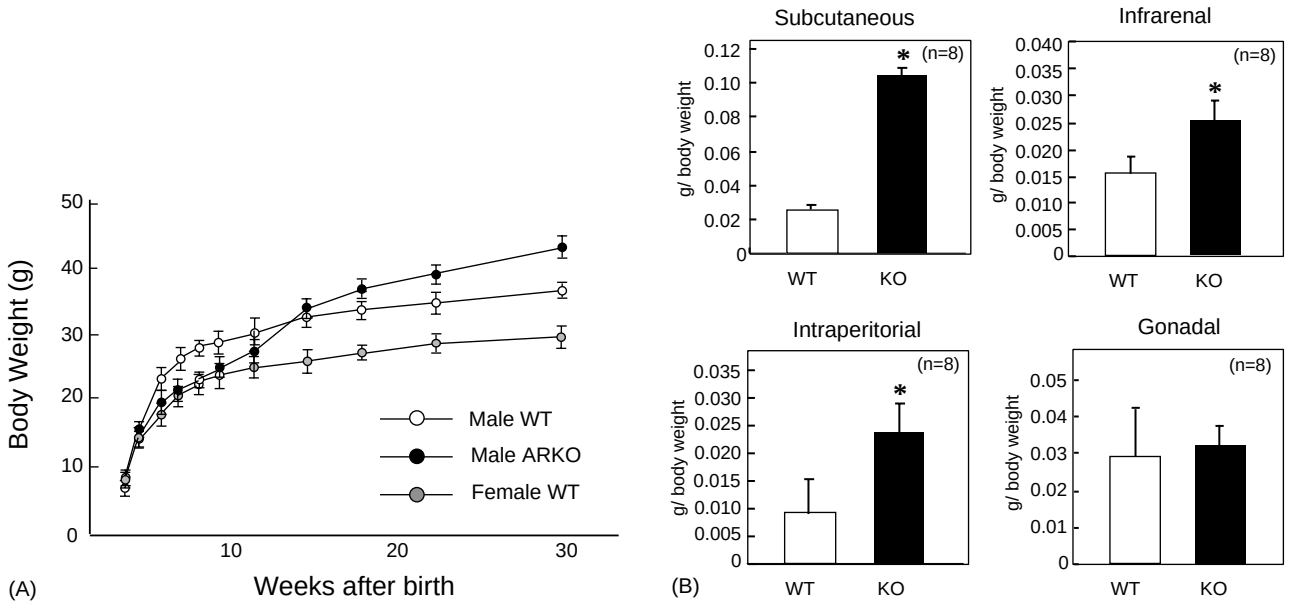

Fig. 2. Obesity in adult male ARKO mice: (A) growth curve of ARKO mice; (B) wet tissue weight of adipose tissue of male ARKO mice (Sato et. al [13]).

sequences in the same direction. Thus, in the Cre transgenic female mouse ($CreTg^+$), one of the two AR gene has been disrupted to generate the female $CreTg^+$ mice with heterozygous disruption of the AR gene. When the male floxed AR mice and these female $CreTg^+$ mice were mated, the AR gene was disrupted by expressed Cre under the CMV strong promoter during the embryogenesis.

The male ARKO which looks like a complete female had the small testes and cecum-like vagina but had no uterus and ovaries; and showed a similarity with the clinical Tfm. [13] The histological findings such as the hypertrophic Leydig cells suggested impaired spermatogenesis. The growth curves for 56 days after the birth of the female ARKO mouse (Fig. 2) were completely comparable with those of the WT female but those of the male ARKO were clearly retarded in comparison with those of the WT male and were rather similar to those of the females.

Estimation of plasma hormone levels in the male ARKO revealed markedly lowered androgens as well as a luteinizing hormone, but there was no difference in the estradiol level in comparison with that of the wild type (WT). These suggest that we can investigate the effect of androgens independently by using the ARKO mouse in that only the AR is disrupted while the estrogen receptors remain intact.

The bone densitometry showed a marked osteopenia, and the 3D-CT indicated that both of the trabecular bone and cortical bone volumes were remarkably reduced in the ARKO male mouse in comparison with that of the WT littermate male mouse at 6–16 weeks of age. Since the bone volumes result from bone remodeling which is the coupling of the formation/resorption of the bone, we compared bone formation and resorption on the proximal tibia in the ARKO and WT male by means of an histomorphometric analysis. Unexpectedly, the bone formation in the ARKO male exceeded that of the WT male by 15–20% (Fig. 3). On the

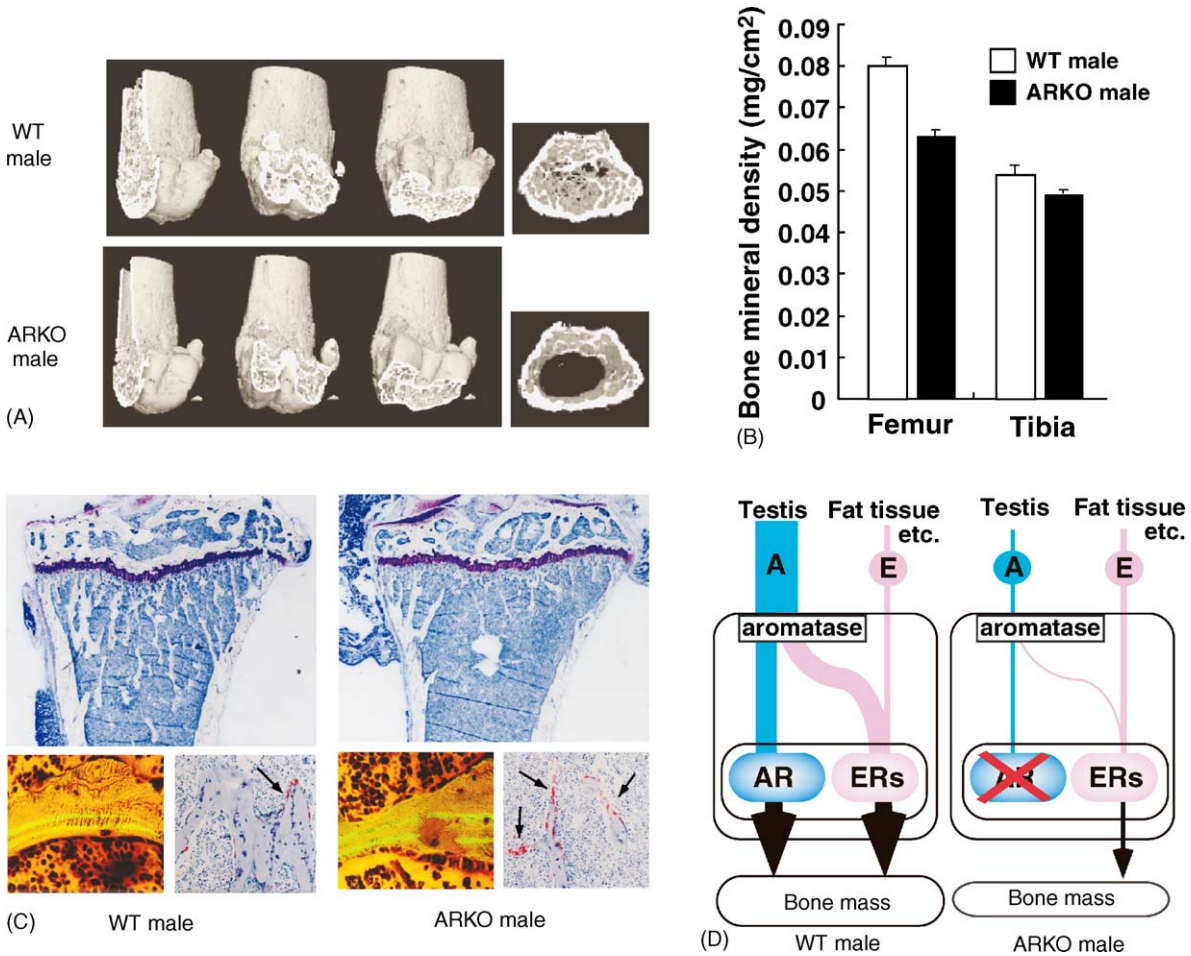

Fig. 3. Osteopenia in male ARKO mice: (A) three-dimensional CT images of distal femora and axial sections of distal metaphyses of male ARKO mice; (B) bone mineral density of male ARKO femur and tibia; (C) high turnover of male ARKO bone. Histological features and histomorphometry of the proximal tibiae from 8-week-old male ARKO and WT mice. For Villanueva-Goldner staining of sections from representative ARKO and WT male littermates, mineralized bone is stained green; (D) schema of skeletal sex hormone action. In male WT mice, skeletal sex hormone activities are mediated by both AR and ER. In female WT mice, skeletal function of ER is likely to dominate over that of AR as serum levels of AR ligands in females are quite low. In male ARKO mice, testicular testosterone production is severely impaired by hypoplasia of the testes, leading to a lack of skeletal sex hormone actions. In contrast, female ARKO mice may not be greatly affected by disruption of AR signaling.

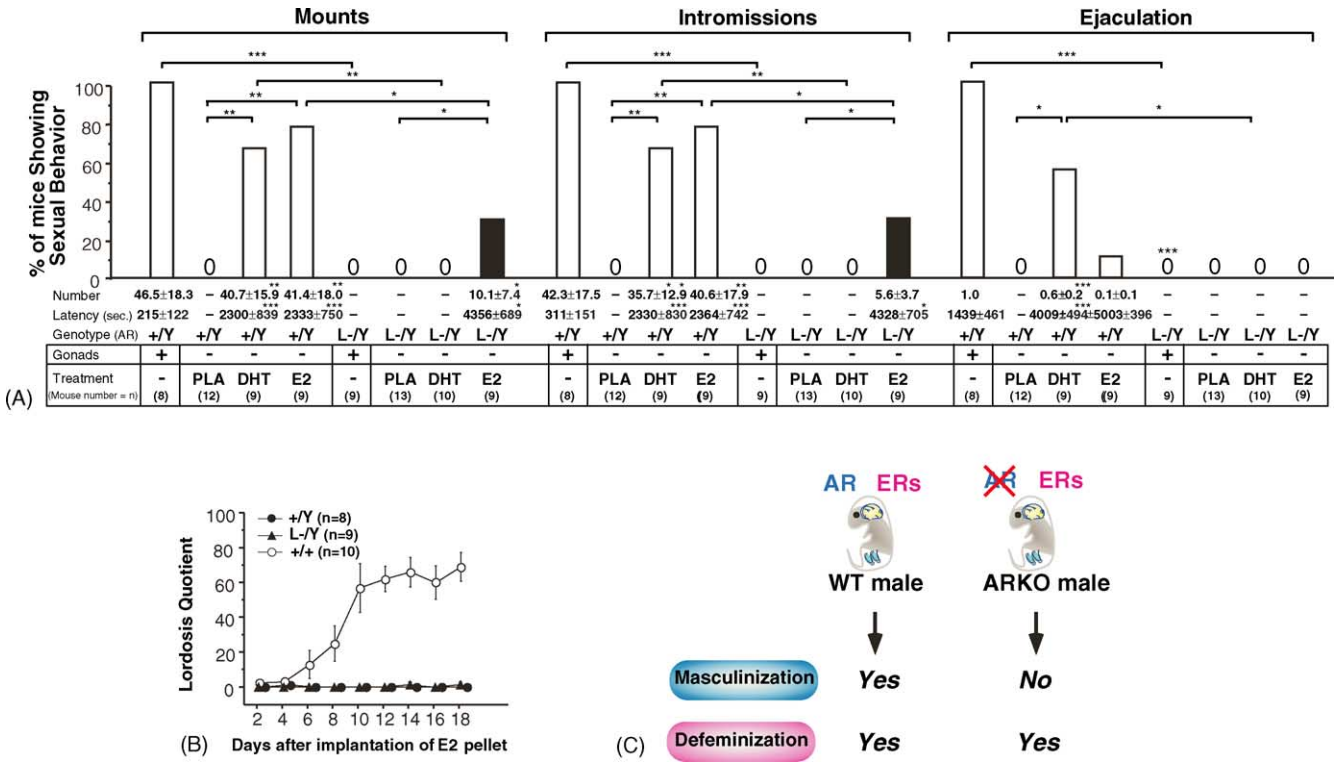

Fig. 4. Male-typical behaviors were impaired in male ARKO mice: (A) impaired male sexual behaviors in ARKO mice were partially recovered by estrogen, but not by androgen; (B) no female sexual behavior (lordosis) in male ARKO mice; (C) brain masculinization requires AR function.

other hand, the bone resorption in the ARKO male was more remarkable and exceeded that of the WT male by 40–50%. In view of these results, we concluded that the reduction in the bone found in the ARKO male was based on the high bone turnover osteopenia [14].

A characteristic change was seen in the body fat composition [13]. More than 10 weeks after the birth ARKO male became fat and the weight exceeded the normal growth curve; and the accumulation of white fat which almost doubled in comparison with the WT male was recognized under celiotomy (Fig. 2). Since there were no clear differences in serum lipids, especially in total cholesterol and free fatty acid, the AR might have suppressed the differentiation of the adipose cells. On the other hand, the sexual behavior of the ARKO mouse either as male or female was found not to be normal; nevertheless the normal gonadal differentiation was found in the ARKO female. Thus, it was considered that abnormal sexual behavior resulted in lowered number of offspring by about half of that of the WT female.

Male-typical behaviors were abolished in male ARKO mice, however, these mice showed no female sexual behavior. Estrogen treatment was effective to recover the impaired male sexual behaviors except ejaculation, suggesting that both of androgen and estrogen signalings mediated their nuclear receptors are essential for expression and maintenance of male sexual behaviors (T. Sato and T. Matsumoto, unpublished result) (Fig. 4).

3. Functional analyses of polyQ-expanded AR mutant in drosophila fly-eye model

An important disease group other than the testicular feminization mutation (Tfm) and androgen insensitivity syndrome (AIS) that is related to the mutation of the AR gene is the triplet repeat disease, or so-called polyQ expansion, in which the poly Q repetitions of the A/B domain of the N-terminal are expanded [4,5]. Spinobulbar muscular atrophy (SBMA) is one of the polyQ diseases and also named as Kennedy's disease. Other polyQ diseases such as Huntington's disease, spino-cerebellar ataxia (SCA1), and Machado-Joseph disease are seen both in males and females [15,16], while manifestation of SBMA can not be seen in the female, even if she is a carrier. Since the AF-1 functions of the A/B domain are androgen-dependent, the reason that the disease occurs only in the male was considered to be dependent on the concentration of androgen.

Aiming at proving this theory, we tried to use the Drosophila fly-eye model [17]. As the lifespan of the fly is short, we thought we could quickly obtain the assay results. The fly possesses nuclear receptors [18]. For example, it has the receptors for ecdysone, metamorphotic hormone, and its partner gene, the ultrabithorax gene. The latter is identical to the human retinoid receptor (RXR). Since the ecdysone receptor of the fly functions as a heterodimer, its DNA binding site is considered to be a direct repeat sequence; on the contrary, the DNA binding site of the human steroid

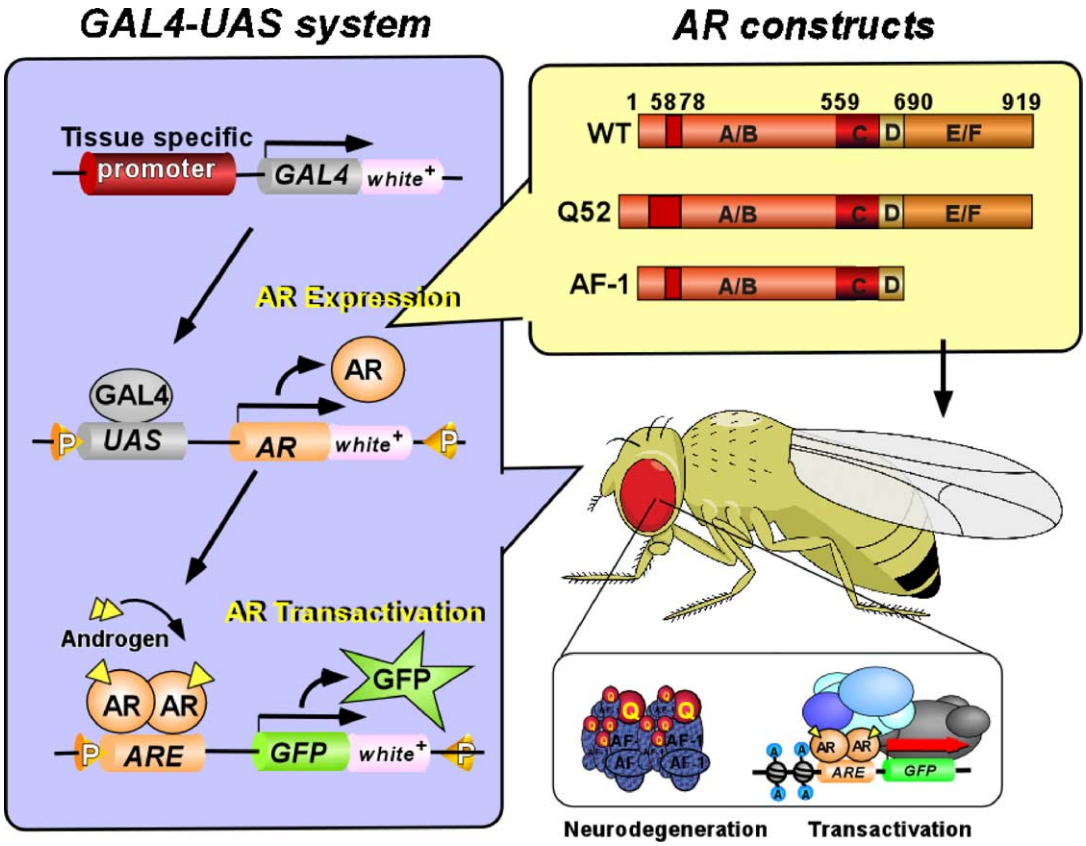

Fig. 5. Inducing hARs to the Drosophila eye, the human ARs, wild type, and polyQ-expanded, were induced in the Drosophila eyes using GAL4 UAS, then the reporter genes were bound to the GFP.

hormone receptor that functions as the homodimer is of a palindrome sequence. Such being the case, we expressed the human AR (hAR) in the fly-eye, tissue/stage specifically, using GAL4 UAS, a conditional gene expression system [19], under the expectation that this AR expression would not impair the functions of the intrinsic receptors in the fly. Then, the reporter gene, a DNA sequence, which can bind to the marker green fluorescent protein (GFP), was bound to the GFP (Fig. 5). In such a fly-eye model, the AR expression can be detected as red by staining it with the antibody; and the transcription function can be recognized as green fluorescent.

Naturally, the human AR has about 20 polyQ repetitions but when we induce too many repeat in the AR, the transcription ability is reduced and also the in vitro protein biosynthesis becomes suppressed. Consequently, we judged around 52 repetitions would be optimal for monitoring the transcription activity and the nerval death. When androgen is fed to the fly that had expressed a wild type AR (ARwt), a green fluorescent is shown in the eye without any abnormal changes. But when the polyQ repeat AR is expressed, the optical nerves (photo-receptor neurons) of the fly are devastated unless the androgen feeding is discontinued; which means the nervous system disorders are androgen-dependent. When cartinostatic agents for prostatic cancer such as hydroxy flutamide and bicalutamide are administered concomitantly, the nerve disorders of the fly were rather worsened. The results justify the development of a new-type anti-androgen for the treatment of prostatic cancer. As the AR is expressed in the nuclear and disrupts the optical nerves while keeping the transactivations, it was clarified that the disorder is based on an intranuclear event; and we recognized an androgen-dependent apoptosis was concurrently taking place.

Fig. 6 illustrates a speculation on the ligand-dependent structural alterations of the polyQ-expanded hAR [17]. The hAR that is inactive in the transactivitiy without ligand (androgen) gains transactivities under the existence of androgen by its structual alterations and also by recruiting co-activators [7,20]; while, the polyQ repeat induce apoptosis by their aggregating property. Since the plasma testosterone is much lower in the female patients (1/20–1/30), in comparison with those of the male patients, the polyQ aggregation may be difficult to occur. On the other hand, most androgen antagonists inhibit the transactivity of the AR by inhibiting recruitment of the co-activators; but they may not induce a structural alteration of the AR that deprive the aggregation by polyQ repeat. Adding finally, most of the polyQ diseases including Kennedy's disease are of late onset; and the disorders in the gonadal function and skeletal muscles appear after middle age. And on the other hand, the sensitivity of the fly-eye in expressing the polyQ repeat AR slightly changes depending on the stage.

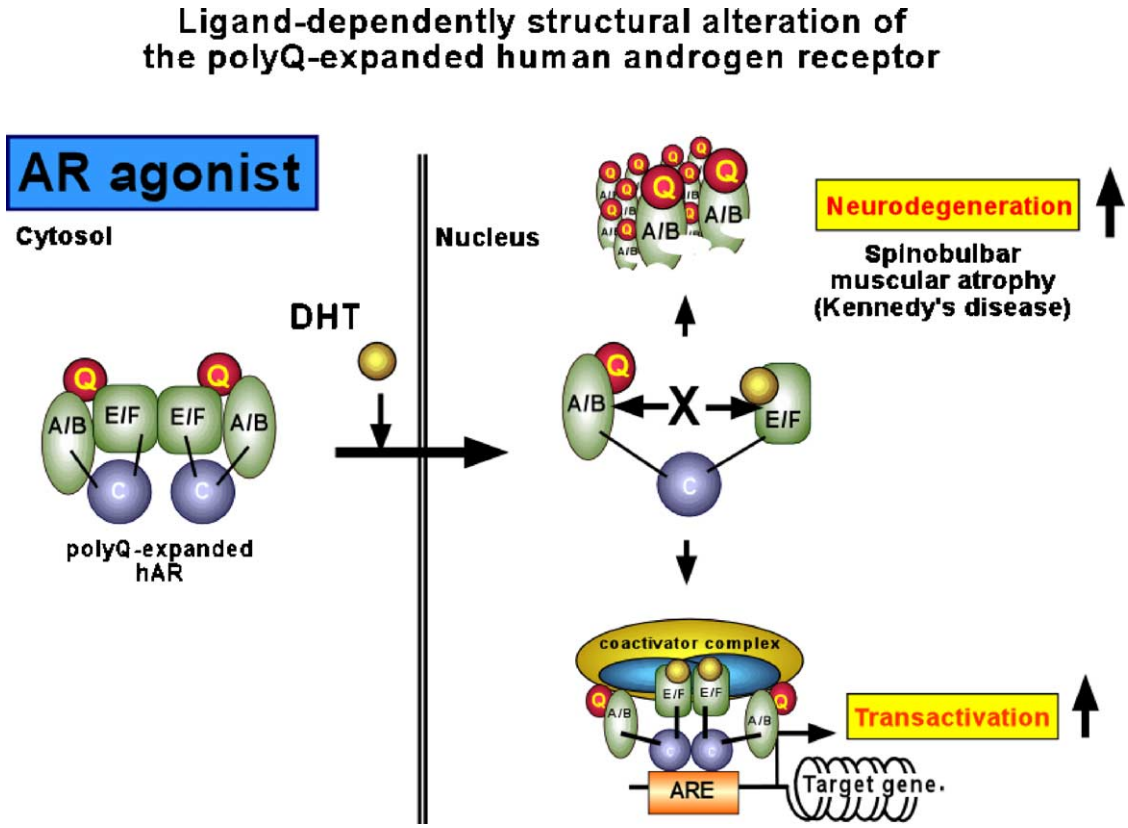

Fig. 6. Androgen-dependent structural alteration by the polyQ-expanded hAR. It is considered that the polyQ-expanded AR is inactive in the transactivation without the agonists (androgens); but under the existence of the agonists, it alters the molecular structure and also recruits the co-activators, while the polyQ repeat induces apoptosis by their aggregation.

In view of these results, we consider that for the management of Kennedy's disease, an anti-androgen treatment, such as an orchidectomy or the development of a new ligand that induces a structural alteration of the polyQ-expansion, may be required.

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