Prostate tumor progression in the TRAMP mouse: Protective effects of the aryl hydrocarbon receptor

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Introduction

The developing male reproductive system is highly sensitive to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[1]. TCDD binds to the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor, to produce sustained alterations in gene expression. Mice lacking the AhR (AhRKO, *Ahr*$^{-/-}$) have permitted further characterization of the role of the AhR in mediating TCDD effects and revealed a physiological role for the AhR in normal development[2-4]. We previously demonstrated that *in utero* and lactational TCDD exposure significantly reduced ventral, dorsolateral and anterior prostate weights, and that these effects were dependent on the AhR[5]. However, reductions in prostate lobe weights in untreated, AhRKO mice compared to wild-type counterparts at various ages demonstrated that the AhR signaling pathway is involved in normal development of the dorsolateral and anterior prostates, but apparently not the ventral prostate. Unaltered serum testosterone concentrations and modest reduction in serum 5α-androstane-3α,17β-diol concentrations could not account for reductions in prostate weights in mice lacking AhR (*Ahr*$^{-/-}$). Normal histology and lack of alteration in androgen receptor mRNA levels further indicate that the reduction in prostate weights is not a result of reduced androgen action in AhRKO mice. The observation that regulation of early prostate growth in mice occurs following AhR activation by TCDD, as well as by loss of AhR, suggests that the AhR may also regulate aberrant prostate growth that results from "reawakening" of the prostate growth regulatory signals later in life[6]. Our objective was to determine if the AhR signaling pathway has an effect on prostate cancer development.

Materials and Methods

Animals and treatment

AhRKO mice developed in the Bradfield lab[3] were backcrossed to C57BL/6J mice for 15 generations. Transgenic adenocarcinoma of the mouse prostate (TRAMP) mice (Jackson Labs) that originated in the Greenberg lab[7-9] were also on the C57BL/6J background. Heterozygous TRAMP (+/-) mice with *Ahr*$^{+/+}$, *Ahr*$^{+/-}$ or *Ahr*$^{-/-}$ genotypes were generated by crossing *Ahr*$^{+/-}$ TRAMP+/+ males with *Ahr*$^{+/-}$ TRAMP-/- females. Initial observations were made by euthanizing litters when prostate tumor burden became excessive in one or more siblings. In the second phase, TRAMP mice of each *Ahr* genotype were necropsied at predetermined times (35, 70, 105, 140, 175 and 210 days of age). At each age tumor incidence was assessed, and tumors and tumor-free prostates were weighed and analyzed using standard histological methods.

Gene expression profile analysis

Two *Ahr*$^{+/-}$ TRAMP+/- male mice with small poorly differentiated nodules were euthanized on postnatal day 140. Small, pale tumor nodules less than 1 mm in diameter without any evidence of vascularization were identified using a dissecting microscope. These nodules represent the earliest possible poorly differentiated TRAMP tumor lesion. Nodules were excised from the surrounding ventral prostate tissue. Two cRNA preparations were generated from each mouse: one from a ventral prostate tumor nodule and the other from non-nodular tissue in the matching ventral prostate lobe. cRNAs were hybridized to Affymetrix MG U74A GeneChips and gene expression signals were calculated with Affymetrix Microarray Suite 5.0 software. Gene expression data were filtered for genes designed as "present" in both nodule arrays or both non-nodular arrays. Paired comparisons were performed between nodule gene expression and non-nodular gene expression in each animal and expression data sets were further filtered for genes whose expression was enriched or decreased by more than 2-fold in both comparisons.

Immunohistochemical analysis

Immunohistochemistry was performed on *Ahr*$^{+/+}$, *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ TRAMP mouse prostates at different stages of tumor development using antibodies against the TRAMP transgene and gene products selected from microarray analysis. Prostate tissue sections were deparaffinized and rehydrated through graded ethanol. Thirty minute antigen retrieval using microwaved citric acid buffer preceded peroxide incubation. Large T antigen antibody (Santa Cruz Biotechnology, Santa Cruz, CA) staining followed manufacturer's instructions for the LSAB immmunostaining kit (DAKO, Carpinteria, CA). Non-specific binding for the chromogranin A antibody (Zymed, San Francisco, CA) was blocked using 5% goat serum. All primary antibodies were incubated overnight at 4°C in a humidity chamber, followed by horseradish peroxidase-conjugated secondary antibody, and color was developed using Vector (Burlingame, CA) NOVA-Red chromagen.

Results and Discussion

Prostate tumor incidence

Striking differences in prostate tumor development were observed between *Ahr*$^{+/+}$ TRAMP mice and their *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ TRAMP siblings when euthanized at various ages. Only 7 of 101 *Ahr*$^{+/+}$ TRAMP mice had tumors (7%), whereas 71 of 272 *Ahr*$^{+/-}$ TRAMP mice (26%) and 29 of 93 *Ahr*$^{-/-}$ TRAMP mice (31%) had overt tumors. By way of comparison, *Ahr*$^{+/+}$, *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ mice without the TRAMP gene do not develop prostate tumors, even beyond 250 days of age. When present, tumor weights were similar in all AhR genotypes, with the major difference being the age at which tumor development occurred. Histologically, mice that do not develop large tumors have prostatic intraepithelial neoplasia (PIN) and well-differentiated adenocarcinoma lesions, with no differences in the severity of pathological changes among the three genotypes. Although these results were not obtained by systematically euthanizing mice of each genotype at the same ages (many were killed when the tumor burden became too large), they demonstrate that the presence or absence of functional AhR greatly influences whether TRAMP mice will undergo tumor progression.

When examined at 35 day intervals, only one of 76 mice had a prostate tumor by day 70 postpartum (Table 1). By day 105, the tumor incidence was 10% in *Ahr*$^{+/+}$ and *Ahr*$^{+/-}$ mice and 21% in *Ahr*$^{-/-}$ mice. On postnatal day 140, tumors were detected in prostates of mice from all genotypes, although the incidence was significantly greater in *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ mice than in *Ahr*$^{+/+}$ mice. Greater tumor incidence was observed for *Ahr*$^{+/-}$ and *Ahr*$^{-/-}$ animals compared to *Ahr*$^{+/+}$ mice up to 210 days of age. *Ahr*$^{+/+}$ mice never developed greater than a 20% tumor incidence, while incidence for *Ahr*$^{+/-}$ mice approached 50%. Approximately 73% of *Ahr*$^{-/-}$ mice developed prostate tumors by 175 days. The relatively low incidence of prostate tumors in our *Ahr*$^{+/+}$ TRAMP mice was below what has been previously reported in TRAMP mouse studies[7, 8, 10, 11], however, those investigations typically utilized a cross between C57BL/6 and FVB mice. In our lab, C57BL/6J x FVB TRAMP mice have greater tumor incidence at postnatal days 105 and 140 than C57BL/6J TRAMP mice. The protective effects of the AhR on tumor incidence are similar to that observed for C57BL/6J mice (data not shown).

Table I. Effect of AhR genotype on prostate tumor incidence in C57BL/6J TRAMP mice

Genotype	Age (days)					
	35	**70**	**105**	**140**	**175**	**210**
Ahr $^{+/+}$	0/20	0/12	1/10	2/18	3/16	1/23
Ahr $^{+/-}$	0/32	1/42	3/40	19/54*	18/41	8/33*
Ahr $^{-/-}$	0/22	0/22	3/14	10/21*	8/11**	3/4**

* Significantly different than *Ahr*$^{+/+}$ at the same age ($p < 0.05$)
** Significantly different than *Ahr*$^{+/+}$ and *Ahr*$^{+/-}$ at the same age ($p < 0.05$)

Large T antigen expression

Although PIN and well-differentiated adenocarcinoma lesions are present in *Ahr*$^{+/+}$, *Ahr*$^{+/-}$, and *Ahr*$^{-/-}$TRAMP mice, we wanted to exclude the possibility that differences in tumor progression in TRAMP mice lacking one or both *Ahr* alleles are not an artifact of altered large T antigen transgene expression, which is required for tumor development. Large T antigen staining was seen in nuclei of epithelial cells lining glands with and without tumors, as expected based on previous reports[7]. Abundant localization was also observed in small nodules, large tumors, and lymph node metastases. Localization was similar in prostates at all stages of tumor development, regardless of AhR genotype. This suggests that the altered tumor development in TRAMP mice of different AhR genotypes is not a result of altered transgene expression. Furthermore, there was no effect of AhR genotype on histological identification of PIN and well-differentiated adenocarcinoma lesions in prostates of each AhR genotype, reflective of transgene action in TRAMP mice. The absence of altered transgene expression or incidence of precancerous lesions or microscopic cancer suggests that the AhR does not affect prostate tumor initiation in the TRAMP model. Rather, it appears that differences in tumor incidence in mice lacking one or more *Ahr* alleles occur during the progression phase of tumor development.

Genes altered during prostate tumor progression

Since the AhR strongly influences prostate tumor progression in TRAMP mice, we sought to identify genes that are differentially expressed during tumor progression, given that these genes may be regulated by the AhR. The gene expression profile of tumor nodules from the earliest identifiable stage of tumor growth in TRAMP mice was similar to the profile for a neuroendocrine phenotype reported previously in cryptdin2-SV40 large T antigen transgenic mice[12]. GeneChip analysis demonstrated that neuroendocrine differentiation in TRAMP mice occurred during

progression from precancerous lesions into poorly-differentiated tumors. mRNA expression of chromogranin A, a neuroendocrine marker, was substantially elevated in TRAMP mouse ventral prostate nodules compared to corresponding non-nodular tissue. Immunohistochemical analysis demonstrated that chromogranin A was not detected in prostates with well-differentiated adenocarcinoma, but was abundant in regions of poorly-differentiated carcinoma. This suggests that onset of a neuroendocrine phenotype in TRAMP mice may reflect a critical stage of prostate tumor progression mediated by the *Ahr*. Current investigations are directed to this signaling pathway in an attempt to explain the reduced tumor incidence in mice with one or both *Ahr* alleles.

Conclusion

Large, poorly differentiated prostate tumors infrequently develop in $Ahr^{+/+}$ C57BL/6J TRAMP mice, but tumor incidence is greatly increased in $Ahr^{+/-}$ and $Ahr^{-/-}$ TRAMP mice. As mice of all AhR genotypes age, their prostates develop PIN and well-differentiated adenocarcinoma lesions characteristic of the TRAMP model. However, $Ahr^{+/-}$ and $Ahr^{-/-}$ TRAMP mice progress beyond those lesions to develop large poorly-differentiated tumors far more frequently than $Ahr^{+/+}$ TRAMP mice ($Ahr^{-/-} > Ahr^{+/-} > Ahr^{+/+}$). This suggests that the AhR may be a tumor suppressor in the prostate. Preliminary evidence suggests that neuroendocrine differentiation occurs as well-differentiated lesions develop into larger tumors. This may be the point where AhR regulates tumor progression in TRAMP mice.

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