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13. ABSTRACT (Maximum 200 Words) Androgen receptor (AR) plays a critical role in prostate oncogenesis. Allelic variants of AR, particularly in length of an N-terminal glutamine (Q) tract, are associated with distinct risks of disease. Mutation of AR in tumors may enter into resistance to treatment and androgen independence. Initiation and progression have been difficult to study due to lack of early disease samples and lack of animal models. This has been partly overcome with transgenic mouse tumor models. However, mouse AR differs significantly from human in the N-terminus. In order to critically evaluate the role of the polymorphic glutamine tract in disease, and to identify relevant sites in the N-terminus whose mutation can lead to androgen-independent AR function, we have "humanized" the mouse by converting its androgen receptor gene to the human sequence. We have done this by homologous recombination in embryonic stem cells, introducing AR alleles with 12, 21, or 41 glutamines. Mice bearing the "wild type" allele with 21 glutamines are normal by all indications, including microarray analysis. However, expression of specific androgen target genes suggests subtle distinctions exist. This will be explored in detail by examining tumor progression and by sequencing AR cDNAs from tumors of castrated vs. intact mice.			
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INTRODUCTION

Both the development and the progression of prostate cancer (PCa) depend on genetic and environmental factors that are poorly defined. One thing all tumors share, however, is an initial dependence on androgen for growth [1]. Polymorphisms in androgen receptor (AR) may impact risk of disease, and somatic mutations may affect progression and response to therapy [2-4]. In almost all cases, endocrine therapy is initially successful, but tumors ultimately become androgen-independent and resist further treatment. Despite androgen independence, AR levels in the tumor remain high and the AR signaling pathway is intact, revealing a continued role of AR in the disease process. AR molecular genetics may be informative for two crucial problems in PCa: **1) How do polymorphisms in AR lead to greater risk of disease? 2) How do somatic mutations in AR during tumor growth circumvent hormone ablation?** This project addresses these questions for the human receptor in a transgenic mouse prostate cancer model, allowing initiation, progression and treatment of disease to be integrated experimentally. Elucidating the mechanisms by which genetic variants alter AR action will validate their use as molecular markers in treatment, and, ultimately, may reveal novel targets for therapy.

BODY

Our hypothesis is that **genetic variation in human AR affects the initiation and progression of prostate cancer**. Germline variation may affect initiation or aggressivity of early disease, while somatic mutations may drive androgen-independent growth. To confirm this hypothesis, our first major objective was to replace the mouse AR with human sequences to study their effects in the mouse in general and in a transgenic tumor model (Transgenic Adenocarcinoma of the Mouse Prostate, or TRAMP [5]). An implicit sub-hypothesis is that **AR species differences impact differential PCa susceptibility**. Since Q (glutamine) tract length enters into PCa risk, we proposed to compare varying length Q tract hAR alleles. We also plan to compare mutations arising in androgen-dependent vs. -independent disease, to identify sites correlating with AR function. Our aims underlying the Statement of Work remain as follows:

Aim I. To study the role in PCa of polymorphisms in human *Ar*, *mAr* will be "humanized" by homologous recombination in embryonic stem cells, to create three *h/mAr* alleles differing in glutamine tract length (12Q, 21Q, 41Q). Differences in androgen action (fertility, behavior, molecular markers) and spontaneous prostate cancer will be studied in mice with *h/mAr* alleles.

Aim II. To determine the role of human AR variants on PCa initiation, *h/mAr* alleles will be placed on the TRAMP background for transgene-induced oncogenesis. Effect of the Q tract will be assessed on prostate pathophysiology and gene expression by cDNA microarray.

Aim III. To determine the role of AR variation on PCa progression, spontaneous mutations will be identified in AR cDNAs from castrated (androgen-independent) vs. intact *h/mAr*-TRAMP mice. The effect of mutations will be determined by introduction into ARs for transfection analysis, with and without coactivators. The effect of

mutations on the oncogenic potential of prostate cells will be tested by tumor formation and metastasis in SCID mice.

Each Aim corresponds to a Task within the Statement of Work. We are progressing well, but a bit behind schedule, due in part to difficulties with one of the three Q tract alleles. The 21 and 41 Q h/mAR mouse strains were created with little problem, but ES cells containing the 12 Q allele had a more difficult time going germline. This was probably more a bit of bad luck rather than a biological difference worth noting, as these cells finally have gone germline (fourth ES clone tested) and the resulting mouse strain seems fine. However, it will still take many months to generate enough mice from the cross to TRAMP to generate tumors to compare to the strains with 21 and 41 Q alleles, and thus we will likely request an additional year extension at no additional cost.

As described previously for the 21 Q h/mAR mice, which have now been in existence for nearly two years, the 41 Q homozygous females as well as males show no differences in gross appearance, behavior, or fertility from wild type littermates. Mice of these lineages are being generated for aging studies and to track prostate physiology, over the next two years. Hormone assays for testosterone and estrogen are being performed to test any differences in feedback regulation. Androgen target genes in several tissues are being assessed for the 41 Q mice, as they were for the 21 Q mice, where we found some expressed at higher levels than in wild type mice.

These mice have also been crossed onto the TRAMP background. At the age of ten weeks one cohort (twelve mice) was castrated and another left intact. Tumors from these mice are being collected, either when the tumor becomes greater than one centimeter by palpation, or when the mice are 26-30 weeks of age. AR cDNAs will be analyzed from these tumors for mutations occurring with progression. Tumors of 41 Q allele mice are in the process of being collected over the next few months, but the tumors of the 21 Q mice have all been harvested. While molecular analysis is ongoing, there are some interesting results with respect to tumor progression, described below.

As noted by others working in the TRAMP model, castration (or treatment with flutamide) may delay but does not prevent prostate cancer, for about 70% of the treated mice [6]. However, in about 30% of the mice, a more aggressive disease initiates in the face of androgen ablation. This finding bears remarkable similarity to results of the SWOG trial in which men were treated with finasteride [7]. Tumor progression is thus heterogeneous in TRAMP mice as it is in men, despite the genetic homogeneity of the mouse model. When TRAMP mice bearing the 21 Q h/mAR gene were tested, we found no remarkable differences in disease progression in intact wild type vs. humanized mice. However, in the castrated group, the humanized mice showed a significant increase in aggressive disease. This is based upon mice requiring euthanasia earlier than the 29 week experimental end-point, which was 2 out of 10 mice for the wild type group compared to 5 out of 13 for the humanized mice. Molecular analysis of AR mutations arising in these tumors is currently underway.

In sum and in accord with our original plan, the second year of this project has largely been spent in establishing the third mouse strain for analysis, characterizing the strains at a gross level, and generating and collecting tumors from the TRAMP cross.

These lines in fact function as predicted, in being physiologically normal but having subtle phenotypes when hormonal effects are examined in greater detail. We are particularly excited by the apparently great increase in aggressive disease with the humanized allele, as we would predict for the stronger human than mouse AR. Thus we are encouraged that these mice will be valuable for assessing the role of androgen receptor in prostate cancer initiation and progression, may provide better subjects for preclinical testing, and may lead to new treatments for disease.

KEY RESEARCH ACCOMPLISHMENTS

- Correctly targeted ES cell lines with 12 Q and 41 Q humanized AR alleles were successfully incorporated into mouse blastocysts, and led to creation of mouse strains containing human AR sequences in place of mouse. These strains are grossly normal and are undergoing further characterization.
- 21 Q and 41 Q humanized AR mice were crossed with TRAMP to generate prostate tumors. The 21 Q tumors have all been collected, and in the cohort that was castrated appear to be a more aggressive form of disease than in wild type littermates, confirming that the human AR gene has a subtly distinct activity or function than the mouse.

REPORTABLE OUTCOMES

We are currently preparing a manuscript detailing the creation and preliminary physiological characterization of the "humanized" AR mouse, but are waiting for the three alleles to be of comparable characterization. We have presented some of the data locally, as well as at the Second International Conference on Prostate Cancer Research, Iowa City, 10/12/02, and at the Interprostate SPORE Conference, San Francisco, 12/3/02. This mouse model will also be used for other experiments proposed as a project within the University of Michigan SPORE in Prostate Cancer, to determine effects of specific antagonists (flutamide, bicalutamide) on incidence of mutations in the humanized compared to mouse AR.

CONCLUSIONS

Mouse strains carrying human rather than mouse AR sequences have been constructed. This will allow direct testing of the role of AR glutamine tract variation in initiation of prostate cancer, which may help clarify contradictory results from epidemiological studies. Further, tumors initiated by transgenes in these mice will allow tracking the role of AR, and AR mutants, in resistance to antiandrogen therapy and androgen independent growth. The site of mutations in human AR sequence may lead to downstream interacting proteins that will be novel and more effective targets in new treatment strategies.

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APPENDICES - none