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13. ABSTRACT ***(Maximum 200 Words)***
Mutations in the AR, changes in growth factor signaling pattern or amplification of the AR may be responsible for androgen independent prostate cancer (AIPC). The aim of this project was to look for changes in the AR in the tumors of patients with AIPC. Due to poor preservation of DNA and low frequency of the AR mutations in available samples I studied two different set of samples. Tumors from patients with prostate cancer before and after androgen ablation therapy (AA) and lymph node metastases from patients who did not receive any AA therapy. In this report I describe the identification of three AR mutants. S863P isolated from lymph node metastases does not bind R1881 and is transcriptionally inactive regardless of the ligands tested. K580R, another lymph node metastatic, DNA binding domain mutant, shows promoter and cell type specific transcriptional activity. Of the 10 patients analyzed before and after AA therapy, one patient showed an expansion of poly-glutamine repeat (from Q20-Q26) following AA therapy. ARQ26 shows reduced transcriptional activity compared to the ARQ20. Future work will include further characterization of the identified tumors and screening of tumors with and without the AR mutations for the AR amplification and activation of MAPK.

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N. Malik 7-27-00

PI - Signature Date

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Introduction:

Prostate cancer is the leading cause of male mortality in the US. Prostate cancer is therefore an active area of research in the field of cancer biology. Growth and maintenance of prostate and prostate cancer is dependent on the presence of circulating androgens and a transcriptionally active AR. Initially, androgen ablation therapy results in regression of tumor, but in majority of cases the tumor progresses from androgen dependent to an androgen independent state within a short time. In androgen independent tumors, the malignant cells continue to express functional AR. The main focus of this project is to develop an understanding of the mechanism by which the tumors acquire androgen independence. Several androgen receptor dependent mechanisms may explain androgen independence. These include 1. Mutations in the AR that allow the receptor to respond to a wider range of hormones. 2. Amplification of the AR so that a low level of hormone is sufficient to provide enough active AR. 3. Changes in growth factor signaling that activates the receptor in an environment of little or no androgen. The aim of my project was to look at metastatic tumors of prostate cancers in patients that have failed androgen ablation therapy. The AR in these tumors is presumably being activated by one of the mechanisms described above.

Annual Summary:

The original statement of works proposed at the outset included two tasks. To analyze tumors from multiple sites in patients who failed androgen ablation therapy and first, to look for changes in androgen receptor and second to perform functional analysis of the mutations identified in the samples, to look for changes in receptor function. Unfortunately, we have had difficulties in obtaining samples with sufficient preservation of DNA. In the absence of those samples, I decided to study two sets of tumors. Metastatic tumors isolated from patients that have not undergone any androgen ablation therapy and a set of tumors obtained before and after androgen ablation therapy. To this end I have looked at quite a few samples in collaboration with Dr Dolores Lamb and Dr Marco Marcelli and have been engaged in characterization of three different mutants.

The detection of mutations was to be performed by single strand conformation polymorphism (SSCP) and any aberrant pattern was to be analyzed by direct sequencing for the identification of mutations. However, with the availability of the automated DNA sequencer that can analyze large number of

samples, the need to look for SSCP was considered superfluous. At the moment, the samples are being analyzed by direct sequencing only.

As proposed in the proposal, I did perform analysis of some of the tumors, which meant isolation of DNA from the tumor samples, amplification of the AR by PCR and then sequencing of the amplified product. But I along with my post-doctoral advisor, Dr Weigel feel that repetition of the same technique (identification of mutations in the tumor samples) does not have any training value and therefore a technician in the lab of Dr Lamb is performing that task. Following the identification of mutations, functional characterization of the mutant receptors is performed by me.

The following is a brief description of some of the AR mutants currently under investigation.

1. **Expansion of poly-glutamine repeats following androgen ablation therapy:** In a screening of 10 patients, performed in Dr Marcelli's lab, cancer specimens were obtained before and after androgen ablation therapy. One patient was found to have an expansion of poly-glutamine repeats from 20 (100% of the specimen) to 26 (70% of the specimen). Initial analysis performed in Dr Marcelli's lab showed that AR Q26 translocates to the nucleus and binds ^{3}H-DHT with affinity equal to that of ARQ20. I am currently examining the effect of this expansion of glutamine repeats on transcriptional activity. My initial results show that ARQ26 is transcriptionally less active than ARQ20 when a simple promoter like GRE is used. Further analysis on more complex promoters is being performed at the moment to further determine the effect this expansion on transcriptional activity of AR.

2. **Lymph node metastatic AR mutants:** In a screening performed in Dr Lamb's lab, where metastatic tumors were isolated from patients that have not undergone any androgen ablation therapy, a hormone binding domain mutant and a DNA binding domain mutant was isolated. Some of the initial results of the functional analysis of these mutants are described below.

S863P: In this mutant, the serine residue at position 863 is changed to proline. I have found that this mutant does not bind R1881 and is transcriptionally inactive regardless of the ligands tested, these include R1881, testosterone, dihydrotestosterone, and estradiol. This mutant is also unable to be activated in a ligand independent manner.

[redacted]

K580R: This is a DNA binding domain mutant where lysine at position 580 is changed to arginine. Initial experiments to test the transcriptional activity of this mutant show that the transcriptional activity of mutant AR is both cell line and promoter dependent. In COS-1 cells, the transcriptional activity of K580R is lower compared to wild type when the simple GRE consensus sequence as well as the MMTV promoter was used. K580R however, is unable to activate the probasin promoter in these cells. In PC-3 cells on the other hand, the probasin promoter is activated at levels comparable to the MMTV or GRE promoter. This difference in transcriptional activity may be due to either a difference in DNA binding or an altered interaction of cofactors with the AR. Future studies on K580R will include a wider range of promoters, and *in vitro* and *in vivo* tests of DNA binding.

Inhibition of transcriptional activity by the AR: In addition to activating transcription through binding to DNA, the AR interacts with other transcription factors and modulates their activity. The proteins belonging to the family of NFκB is among them. I have established assays for measuring the effect of the AR on Rel A dependent transcription as well as of Rel A on the AR dependent transcription. It will be interesting to study the effect of mutation in the AR on the transcription genes involved in cell proliferation.

Because of the difficulty in obtaining good quality samples and the relatively low frequency of the AR mutations, I have changed my research plan slightly. I will take a subset of samples from patients that have failed androgen ablation therapy. This set of samples will consist of those AR that have been identified as having mutations, as well as some that have no mutations and will determine, as originally planned, whether the AR is amplified or the MAPK is activated. In several patients, Dr Lamb has detected mutations identical to the LNCaP mutation. These samples will be included in my study. I would predict that the altered hormone binding of this mutant AR is sufficient to induce androgen independence and that I will see the AR gene amplification and/or MAPK activation more frequently in tumors that do not exhibit this mutation. I will continue with the functional analysis of the mutants identified in untreated metastases.

Key accomplishments:

- Learned techniques used in processing of tumor samples such as isolation of DNA, PCR amplification of the AR, sequencing of the AR.
- Isolation of the AR mutants from patients with metastatic disease.
- Partial characterization of the isolated mutants.

Reportable outcomes:

This work described in this report was presented at the annual Endocrine Society meeting in June 2000. The abstract is enclosed.

I was awarded a travel award by Women in Endocrinology to present this work at the meeting.

Androgen receptor mutants identified in prostate cancer metastases that exhibit reduced activity.

N P Malik[1*],AJ James[1], M Marcelli[1,2], DJ Lamb[1,3], and NL Weigel[1]. Molecular and [1]Cellular Biology, Baylor College of Medicine, Houston Tx 77030; [2]Medicine, Baylor College of Medicine, Houston Tx, 77030; and [3]Urology, Baylor College of Medicine, Houston Tx, 77030.

The role of androgen receptor mutations in prostate cancer has been a subject of much debate. Androgens stimulate the growth of prostate tumors and tumors from patients who have failed flutamide therapy sometimes express mutant androgen receptors that respond to flutamide as an agonist. In normal prostate, androgens induce expression of stromal cell growth factors that stimulate the growth of the epithelial cells. Androgen action in the epithelial cells induces synthesis of proteins characteristic of the differentiated state such as PSA. In the last few years, we have identified a number of androgen receptor mutations in lymph node metastases of prostate tumors from patients who have not previously undergone treatment. To date, our functional analyses show that the majority of the mutations that exhibit a phenotype, show reductions in either hormone dependent or ligand independent activity. Mutations have been detected both in the DNA binding domain and in the hormone binding domain. One previously described mutant C619Y, fails to bind to DNA and is transcriptionally inactive. Two other DNA binding domain mutants, A586V and A587S appear to show no change in transcriptional activity whereas a third, T575A shows enhanced activity. The other mutations are located in the hormone binding domain and exhibit a wide variety of phenotypes. Q919R responds reasonably well to ligand but has lost its capacity to be activated by forskolin, an activator of Protein Kinase A. V757A exhibits normal hormone binding, but its transcriptional activity is specifically reduced in prostate cancer cell lines. S863P does not bind hormone; it is transcriptionally inactive either in response to hormone or to forskolin. Preliminary data show that A748T also has reduced activity. These studies suggest that reduced androgen receptor activity early in the progression of prostate cancer may allow the epithelial cells to dedifferentiate and resume proliferation.

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DEPARTMENT OF THE ARMY
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