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TITLE: ATM Heterozygosity and the Development of Radiation-Induced Erectile Dysfunction and Urinary Morbidity Following Radiotherapy for Prostate Cancer

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CONTRACTING ORGANIZATION: Mount Sinai School of Medicine
New York, New York 10029-6574

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| E-Mail: | jamie.cesaretti@msnyuhealth.org |

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The goal of this training grant project is to determine whether the prevalence of ATM carriers among prostate cancer patients treated with radiotherapy that develop erectile dysfunction and urinary morbidity is greater than the prevalence of ATM heterozygosity among patients that do not develop this complication. Regardless of the scientific outcome of the proposal the PI will be left with a vast experience in translational research from which to form new hypotheses and research strategies as he begins his career as an independent physician scientist. To assure a well-rounded experience, the school of medicine will insure that the PI will participate for the first two years of the funded period in Mount Sinai’s rigorous clinical research training program. The NIH sponsored program will give the PI formal instruction in Clinical Research and Policy Evaluation, Epidemiology and Biostatistics, Basic Science for the Clinical Investigator, Cultural, Illness, and Community Health Outcomes, Behavioral Medicine, and Ethical Issues in Clinical Research. Also the PI, while at Mount Sinai, will make significant progress in establishing collaborative relationships with well-established prostate cancer researchers and will continue this approach in order to expand the scope of the outlined proposeral through the funding period of this grant.

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Radiotherapy, Mutation Screening, Functional Analysis

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Introduction:

A significant proportion of prostate cancer patients treated with radiotherapy develop erectile dysfunction and urinary morbidity induced by exposure to a high dose of radiation. In some cases there are explanations for these reactions, such as doses to large volumes of normal tissue or pre-existing medical conditions such as diabetes or collagen vascular diseases. However, there exists an important subset of patients with no clear explanation for excessive post-treatment morbidity and the potential for a genetic basis must be considered. The purpose of this study is to investigate whether the ATM gene plays a role in this radiation sensitivity. This gene was selected, as the protein it encodes, plays a critical role in the response of cells to irradiation and the repair of radiation-induced damage. Furthermore, cells possessing one mutated copy of this gene are radiosensitive. In addition, the results of a pilot study screening breast cancer patients are supportive of the hypothesis that patients who are carriers of an ATM mutation are more likely to develop radiation-induced complications.

The principal goal of this project is to determine whether men who inherit a mutated copy of the ATM gene are more prone to the development of radiation-induced erectile dysfunction and urinary morbidity. This will be accomplished through comprehensive screening of the ATM gene for germline mutations. If a correlation is found between radiosensitivity and ATM heterozygosity, this would indicate that possession of a mutated copy of the ATM gene results in susceptibility to complications for prostate cancer radiotherapy patients. In addition, a determination will be made as to the pathogenic consequences of each ATM mutation through the use of functional studies that will examine the ability of the ATM protein to act normally in cells from patients who are carriers of a mutation in this gene. This project represents the first study to use the powerful DHPLC mutation screening technique to investigate the association between possession of a mutated ATM gene and both erectile dysfunction and the entire clinical course of a patient’s urinary morbidity after treatment with radiation for prostate cancer. It is also the first study to examine whether there is a correlation between the presence of a mutation, development of a radiation-induced complication, and impairment of ATM protein function based upon cellular and molecular analyses.
My annual report covers the period from 2/1/05 to 1/31/06. I will successfully complete the Mount Sinai Clinical Research Training Program, which is sponsored by an NIH K30 Clinical Research Curriculum Award. In addition to the training plan regarding the Clinical Research Training Program I have completed additional coursework offered by Mount Sinai will be conferred a masters degree in Clinical Research in May 2006. My coursework this year included Clinical Research Thesis Project, Clinical Research Thesis Project Design, Clinical Studies Journal Club I, Clinical Studies Journal Club II, Clinical Research Works in Progress Seminar Series I, Clinical Research Works in Progress Seminar Series II, and Scientific Writing and Presentation.

I have performed DHPLC on 163 men from the Mount Sinai Prostate Cancer Tissue Repository. I am currently finalizing the required PCR work for the group. I have accrued 35 of the expected 50 patients needed for the study who developed erectile dysfunction following brachytherapy. In addition I have accrued 21 patients of an expected 50 with severe urinary morbidity following the brachytherapy. In addition I have also performed DHPLC on 107 patients who did not have either erectile dysfunction nor severe urinary morbidity following the procedure.

I have published my first collaborative publication in association with Jan Overgaard’s group in Denmark. The publication details an analysis of the ATM gene in patient’s with severe radiation side effects following radiotherapy for breast cancer. In addition, I have continued to spend 4 hours with Simon Hall M.D., the chairman of Urology at Mount Sinai; in the Maury Dean Center for Prostate Health. From these meetings I have continued to solidify my research ties with his faculty. I am working with Natan Bar-Chama MD, an expert in the diagnosis and treatment of erectile function, on a prospective study of the use of sildenafil to prevent brachytherapy induced erectile dysfunction. Lastly, my department recruited another physician named Johnny Kao, M.D. in July 2005, who has also been awarded a Physician
Research Training Grant from the Department of Defense. We have several protocols and collaborative projects which are ongoing.

I have published four articles this year as an author. (see references) In addition, I gave an oral presentation at this year's American Society of Radiation Oncology meeting entitled, “Impact of Low Dose Rate Prostate Brachytherapy on the Sexual Health of Men with Normal Pre-treatment Sexual Function; an Analysis at Seven-years Minimum Follow-up.” I also gave an invited talk at this year's Radiation Research Society meeting at a session entitled, “Update of Normal Tissue Radiobiology in the IMRT Era”; my talk was entitled,” Towards a predictive genetic model of adverse late radiation effects.” These talks were in addition to several other collaborative efforts. (see appendix)

In terms of obtaining additional funding opportunities, I have received funding from the NIH Loan Repayment Program. In addition, in association with my mentor Barry Rosenstein, PhD, work on a study entitled, “ATM sequence variants are predictive of adverse radiotherapy response among African-American men” from the American Cancer Society, continues to progress on schedule.
KEY RESEARCH ACCOMPLISHMENTS:

Completed 18 months of coursework required for Clinical Research Training Program.

Perform PCR with DNA samples isolated from 35 with erectile dysfunction and 21 patients with severe urinary side effects and 75 matched controls obtained from the Mount Sinai Prostate Cancer Patient Tissue Repository.

Completed DHPLC on 163 patient’s obtained from the Mount Sinai Prostate Cancer Tissue Repository. In addition, I have identified all abnormal chromatograms within the sampled group.

I have completed the DNA sequencing of all to identify PCR products that may possess ATM mutations based upon the appearance of aberrant chromatograms.

I have established a research collaboration with Jan Overgaard’s group in Denmark. His group leads European efforts to identify a link between clinical radiation sensitivity and an individual’s genetics.

I presented my findings regarding this project at an invited talk at the Radiation Research Society’s annual meeting in Denver Colorado, 10/18/2005 and at the Annual American Urological Association in San Antonio, Texas 5/2005.

I have obtained funding from the National Institutes of Health under the Loan Repayment Program. My initial funding period will be from 7/1/2005 to 6/30/2007.
REPORTABLE OUTCOMES:

Publications:


Presentations:


Cesaretti JA. “Intensity Modulated Radiation Therapy for Prostate Cancer” and “Combined Modality Therapy for Prostate Cancer.” Advanced Workshop in the Treatment of


**Cesaretti JA, Stone NN, Stock RG.** “Impact of Low Dose Rate Prostate Brachytherapy on the Sexual Health of Men with Normal Pre-treatment Sexual Function; an Analysis at Seven-years Minimum Follow-up.” ASTRO 47th Annual Meeting, October 2005, Denver, Colorado. (Oral Presentation)


CONCLUSIONS:

My training grant is progressing on several important fronts. I continue to be ahead of schedule in terms of patient accrual. I have completed the DHPLC work of 164 accrued patient’s to this point. I am nearing completion of PCR necessary to identify significant mutations in the study group. Completion of these initial phases will allow for me to proceed to the planned functional assays in the next few months.

I have expanded my collaborative network and have published my first collaborative paper on the subject of genetic predisposition to side effects as an e-publication on December 9, 2005.

I have received an NIH loan repayment grant.

I have completed three-quarters of the coursework necessary to complete the K30 Physician Research Training Program; In addition, I have done enough coursework to be awarded a Masters degree in May 2006 in Clinical Research.

The results of my research project were presented at both the AUA and ASTRO/RRS national meetings.
REFERENCES:


APPENDICES:

Presentation - ASTRO/RRS invited talk

Article - Andreassen paper

Abstract - Radiation Research Breast

Abstract - ASTRO ATM Breast

Abstract - ASTRO Erectile Dysfunction

Abstract - ASTRO Sexual Health Survey

Abstract - AUA Erectile Dysfunction and ATM

CV
Towards a predictive genetic model of adverse late radiation effects.

Jamie Cesaretti, M.D.
Assistant Professor
Mount Sinai School of Medicine

There is a well known genetic basis for normal tissue radiosensitivity.

Radiation Reaction in Ataxia Telangiectasia

What is radiation sensitivity?

What characteristics might one look for in a candidate gene?

Once one has the gene, which variants should one value?

How important is the clinical data?

What about dosimetry?

What considerations should be made regarding the genetic background of the tested population?
Characteristics of a candidate gene(s).

- Involvement in DNA repair from radiation damage (many many genes)
- Correlation with a previously described radiation sensitivity syndrome (fewer genes and ATM)
- Gene implicated in cancer predisposition (several genes)
- Gene involved in repairing oxidative damage (many genes)
- Cell cycle regulation, chromatin stewardship genes, etc.

There are other candidate genes.

- TGFB1 – multifunctional cytokine causes fibrosis
- SOD2 – encodes important anti-oxidant enzyme
- XRCC3 – homologous recombination of DSB
- XRCC1 – single strand break recombination

What variant is meaningful?

- Common variants will, if positive, offer the most potential statistically (i.e. 10-20% incidence)
- Functional variants – which have the potential of conferring a structural change (most convincing)
- Single nucleotide polymorphisms (SNPs) – would be the most amenable to the development of a commercially viable screening test
- Gene exploration versus screening: The exploration of different populations may change our assumptions about the functional significance of any given polymorphism.
Slide 10

**When is clinical information meaningful?**

- Prospectively collected.
- Long-term follow-up (A cancer patient with a good prognosis).
- Use of common validated toxicity measures.
- The toxicity is easily and reproducibly scored.
- Toxicity is clinically significant.
- Data collector is blinded from genetic analysis.
- Known confounding factors should be identified (tamoxifen, anti-oxidants, amifostine, chemotherapy, familial syndromes).

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Slide 11

**The importance of dosimetry.**

- In order to elicit a difference, patients need to have been treated with a spectrum of high doses (prostate, some older breast regimens, head and neck, lung, sarcoma).
- Dosimetry should be prospectively collected, using 3D appreciations of anatomy.
- Different dose rates may have different implications in analysis.
- Toxicity has to occur in order for there to be a successful association.

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Slide 12

**Populations should be homogeneous.**

- There are racial-ethnic differences between the incidence of SNP’s in the population.
- It may not be the same answer for every ethnic group in terms of at-risk alleles.
- In validating or invalidating genetic associations to radiation toxicity - a detailed description of the genetic background of patients should be apparent.

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ATM is a good candidate gene.  

Associated with a genetic disorder with XRT sensitive component.  

It is involved in DSB repair.  

Other’s have reported radiation sensitivity among heterozygotes.

------

A connection has been made in prostate cancer using EBRT and screening for ATM variations.  

There was an over-representation of diabetes in the sequenced population.

------

Which polymorphisms have functional significance? Why?  

The complexity of analysis required in exploration of a gene can be daunting.

------

ATM: Ataxia Telangiectasia Mutated  


Slide 19

Brachytherapy as a model for developing a predictive test for rectal bleeding.

Very high doses. Variable patient dosimetry. Toxicity does happen frequently. Multiple toxicities can be measured. Patients live to have late effects. Toxicities have clinical meaning.

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Slide 20

Defining the Risk of Developing Grade 2 Proctitis Following I-125 Prostate Implants Using a Rectal Dose Volume Histogram Analysis

![Graph showing the relationship between rectal volume and grade 2 proctitis.](image)


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Slide 21

Others have found similar relationships between dose and rectal bleeding.

![Graph showing the relationship between prostate tissue and rectal bleeding.](image)

Slide 22

Results: Rectal Bleeding

- RTOG grade 1: 4/37 = 11%
- RTOG grade 2: 2/37 = 5%

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Slide 23

Risk of Developing Grade 2 Proctitis when factoring in ATM status.

Effect of Rectal Volume Receiving 160 GY

Slide 24

Does a combined analysis of previously described genetic associations predict late effects with more accuracy than analysis of a single gene?
ATM codon 1853, XRCC1 codon 399, XRCC3 codon 241, SOD2 codon 16 and TGFB1 codon 10, respectively, were defined as putative ‘risk alleles’. The patients were grouped according to the total number of risk alleles they possessed. ED50 values were calculated for patients with 2-3, 4-5 and 6-7 ‘risk alleles’.

**Slide 26**

*Are there significant differences across populations in terms of genetic associations to radiation sensitivity?*

**Slide 27**

**Genetic Predictors of Adverse Radiotherapy Effects in African-American Breast Cancer Patients**

**METHODS**

34 African-American women and 73 non-African American women with a minimum of 2-year follow-up underwent breast-conserving surgery and standard adjuvant radiation therapy for either DCIS or early stage breast cancer between 1990 to 2003.

**CONCLUSION:** Of the 24 ATM variants identified, only 7 were shared between African-American and non-African American women.

*Radiation Research 2005 #107*
Genetic Predictors of Adverse Radiotherapy Effects
The Gene-PARE Project

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Summary:
Each individual may be able to have a genetically determined DVH in the next several years.

This could serve as a rational basis for further dose escalation in order to better compliment the rapid application of technical innovations.

Acknowledgements:
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Alice Ho, M.D.
Grace Fan, M.D.
Chris Peters, M.D.
Kathy Lu, M.D.
CLINICAL INVESTIGATION

ATM SEQUENCE VARIANTS AND RISK OF RADIATION-INDUCED SUBCUTANEOUS FIBROSIS AFTER POSTMASTECTOMY RADIOTHERAPY

CHRISTIAN N. ANDREASEN, M.D., JENS OVERGAARD, M.D., D.M.SC., F.A.C.R., F.R.C.R., JAN ALSENIER, PH.D., MARC OVERGAARD, M.D., CARSTEN HIRSHKO, PH.D., JAMIE A. CESARETI, M.D., DAVID P. ATENCIO, PH.D., SHEERYL GREEN, M.D., SILVIA C. FORMENTI, M.D., RICHARD G. STOCK, M.D., and BARRY S. ROSENSTEIN, PH.D.

Departments of *Experimental Clinical Oncology and 1Oncology, Aarhus University Hospital, Aarhus, Denmark; Department of Radiation Oncology, University of Heidelberg, Mannheim Medical Center, Mannheim, Germany; Departments of 2Radiation Oncology, 3Community and Preventive Medicine, and 4Dermatology, Mount Sinai School of Medicine, New York, NY; Department of Radiation Oncology, New York University School of Medicine, New York, NY

Purpose: To examine the hypothesis that women who are carriers of genetic alterations in the ATM gene are more likely to develop subcutaneous fibrosis after radiotherapy for treatment of breast cancer compared with patients who do not possess DNA sequence variations in this gene.

Methods and Materials: DNA samples isolated from fibroblast cell lines established from 41 women treated with postmastectomy radiotherapy for breast cancer were screened for genetic variants in ATM using denaturing high-performance liquid chromatography (DHPLC). A minimum follow-up of 2 years enabled analysis of late effects to genetic dose–response curves and to estimate the dose that resulted in a 50% incidence of Grade 3 fibrosis (ED50).

Results: A total of 28 genetic alterations in the expressed portions of the ATM gene, or within 18 bases of each exon in regions encompassing potential splice sites, were detected in 22 patients. The ED50 (95% confidence interval) was 60.2 (58.4–63.1) Gy calculated for patients without a sequence variant and did not differ significantly from the ED50 of 58.6 (54.0–63.1) Gy for the group of patients with any ATM sequence abnormality. The ED50 of 53.7 (50.6–57.2) Gy for those patients who were either homozygous or heterozygous for the G→A polymorphism at nucleotide 5557, which results in substitution of serine for alanine at position 1853 of the ATM protein, was substantially lower than the ED50 of 68.5 (67.0–69.4) Gy for patients not carriers of this sequence alteration. This resulted in an enhancement ratio (ratio of the ED50 values) of 1.15 (1.05–1.22), which was significantly greater than unity.

Conclusions: The results of this study suggest an association between the ATM codon 1853 Asn/Asp and Asn/Asn genotypes with the development of Grade 3 fibrosis in breast cancer patients treated with radiotherapy.

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INTRODUCTION

Radiation-induced fibrosis (1) constitutes an important potential complication after radiotherapy (2, 3). The development of late normal-tissue reactions in breast cancer patients receiving radiotherapy shows considerable variation between individual patients. Although dosimetric variation or underlying medical conditions may be partly responsible for the morbidity, this explanation does not account for all differences between patients. Often, the adverse response is simply ascribed to unknown individual variations. However, evidence in support of genetic factors being responsible for interpatient variation in radiotherapy is emerging, such as an examination that was performed of radiation-induced telangiectasia in breast cancer patients (4). This study described a relatively large individual variation in the progression rate to development of telangiectasia for the same radiation treatment. It was concluded that 30–90% of this variability was due to deterministic effects related to the
existence of possible genetic differences between individuals, whereas only 10–20% of the variation could be explained through stochastic events arising from the random nature of radiation-induced cell killing and random variations in dosimetry and dose delivery.

Substantial work has been performed in recent years in an effort to identify radiosensitivity candidate genes as well as the specific single nucleotide polymorphisms (SNPs) and rare genetic variants associated with the development of adverse responses to radiotherapy (5, 6). The first gene ever to have received significant attention was the mutated in axinless inactivation (AT) gene, ATM, as it was reported more than 30 years ago that patients suffering from the disease axinless exhibited unusually severe and devastating responses to ionizing radiotherapy (7, 8). The ATM protein functions primarily as a protein kinase involved in cell cycle checkpoints controlling the cell cycle of tumor cell growth and differentiation. ATM was found to be overexpressed in a number of human cancers (9, 10). However, evidence in support for the role of AT mutant alleles affecting radiosensitivity to breast cancer patients came from a study in which 46 breast cancer patients were screened for ATM sequence mutations. It was reported that 100% (313) of patients tested positive for ATM mutations. A second study reported a significant association specifically between homozygous carriers of the G→A transition at ATM nucleotide 5557 and adverse radiotherapy response (11). In addition, evidence has been obtained demonstrating an association between ATM sequence variants with clinical radiosensitivity in prostate cancer patients (12, 13).

The mutation screening technique used in this study, denaturing high-performance liquid chromatography (DHPLC) (14–17), is a robust technique that can be used to screen any gene in a large population for SNPs, as well as small deletions and insertions. The advantage of DHPLC is that it can be performed in an automated fashion to identify and quantify small changes in DNA sequence variants in a population. Of greatest importance in this study, the technique of DHPLC assesses a sensitivity and specificity for DNA sequence variant detection in ATM approaching 100% (18).

During the period 1978–1980, postmastectomy breast cancer patients were treated in Aarhus, Denmark with a hyperfractionated radiotherapy protocol. Because of the high incidence of late normal tissue complications, the fraction size was reduced to 2 Gy in 1980 (19). As a result, the majority of patients included in the present study received large doses per fraction. Skin biopsies were obtained from the patients, and fibroblasts were cultured (20), thereby providing a source of DNA for genetic analysis. Compared with most patients treated in recent decades who have been given standard radiotherapy protocols using 1.8–2.0 Gy fraction sizes, resulting in modest normal tissue biologic doses and a relatively low incidence of late normal tissue toxicity, this Danish patient cohort represents a unique population because of the relatively large biologic doses received and the availability of skin biopsies. Further-
sites was graded using a four-point scale identical to that later used in the Late Effects of Normal Tissue—Subjective Objective Management Analytic (LENT-SOMA) scoring system (26). Because of the large fraction sizes used for treatment of the majority of the patients, the biologic doses were often relatively high (Table 1). Therefore, Grade 3 fibrosis was detected in 37% of the individual treatment fields examined, with 50% of the patients exhibiting at least one field with this late effect. ATM genetic screening

DNA samples were isolated from skin fibroblast cells using the Puregene DNA Isolation Kit according to the manufacturer’s protocols (Centra Systems, Minneapolis, MN). Polymerase chain reaction was used to amplify each of the 62 exons, and short intronic regions flanking each exon, that comprise the coding region of the ATM gene using primers previously described (18). Dideoxy analysis was performed on a WAVE Nucleic Acid Prog-

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<th>Table 1. ATM genetic status, dose, and fibrosis in each of the 41 patients</th>
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<td><strong>ATM Variant</strong></td>
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*Abbreviation: n/a = not applicable.
* Anterior photon field including supraventricular region and anterior region.
* Anterior electron field.
* The part of the anterior photon field covered by a 5-mm wax bolus.
* Equivalent dose of 2 Gy per fraction.
* G = go fibrosis, 1 = fibrosis.
* T = thymocyte; all other variants were present in the heterozygous state.
RESULTS

Table 1 provides a list of the 26 genetic alterations in the expressed portions of the ATM gene, or within 10 bases of each exon, in putative splice site regions, that were detected in 22 of the 41 screened breast cancer patients treated with postmastectomy radiotherapy. In addition, this table lists the dose given to each field and whether Grade 3 fibrosis developed.

Figure 3 displays the dose–response for patients found to harbor any ATM sequence variant compared with the group of patients who did not possess an ATM sequence alteration. These curves did not differ significantly from each other (p = 0.56). The ED_{50} (95% confidence of interval) was 58.4 (54.0–63.1) Gy for the group of patients with any ATM sequence abnormality and 60.2 (55.7–65.1) Gy for patients without a sequence variant. This corresponded to an enhancement ratio of 1.03 (0.97–1.21). A similar analysis was performed for the patients with two ATM variants (6 patients, including 2 being homozygous for the 5557 G→A polymorphism), compared with those with less than two alterations. There was a trend that the dose–response curves for these groups differed from each other (p = 0.13) (dose–response curves not shown). The E_{50} value for patients with two sequence alterations was 54.8 (51.3–58.5) Gy as compared with 60.5 (56.7–64.5) Gy for those with less than two alterations. The corresponding enhancement ratio was 1.10 (1.03–1.19).

With regard to the 5557 G→A SNP, the dose–response curve for the 7 patients who were either homozygous or heterozygous for the G→A transition polymorphism was significantly different compared with the curve derived from patients without the polymorphism (p = 0.03) (Fig. 4). For these two groups, the ED_{50} values were 55.7 (50.2–57.5) and 60.8 (57.0–64.8) Gy, respectively, and found, leading to an enhancement ratio of 1.13 (1.05–1.22). By contrast, no significant difference was found between the dose–response curve from the 6 patients with the IV362 + 8A/C SNP polymorphism.
Fig. 4. Dose-response curves for subcutaneous fibrosis in patients with either the G→A polymorphism at nucleotide 5557 or not possessing this alteration.

...p≤0.029...

DISCUSSION

Postmastectomy breast cancer patients treated with two different radiation protocols, resulting in a range of 2 Gy equivalent doses from 34–69 Gy in three fields, were screened for genetic alterations in ATM. Statistically significant results were obtained when the patients were analyzed with respect to the possession of the 5557 G→A SNP. Regarding the possession of two ATM sequence variants, a statistically significant result was found when the analysis was based on the ED_{50} estimates and enhancement ratios provided by logit analysis, whereas only a trend toward significance was found when the dose–response curves were compared by logistic regression. For these two groups, enhancement ratios of 1.13 and 1.10 respectively were found. A further analysis revealed a high degree of concordance between the group of patients with two sequence alterations and those harboring the 5557 G→A SNP (5 of 6 patients with two alterations had the 5557 G→A SNP and 5 of 7 patients with the 5557 G→A SNP had two alterations) (Table 1). Based on these observations, it seems plausible that the enhanced fibrosis risk observed among patients with two alterations was mediated by the possession of the ATM 5557 G→A SNP. Thus, the results suggest that women who were carriers of the 5557 G→A polymorphism developed Grade 3 subcutaneous fibrosis at lower doses compared with patients who did not possess this type of genetic alterations. In contrast, the findings of this work do not support an association between the development of fibrosis and any other ATM variant detected in the group of patients screened. However, we emphasize that this study provided limited statistical power to detect associations for alterations with low carrier frequencies.

Although multiple comparisons were made in this study, a Bonferroni correction (30) was not applied to the calculated p-values, as the purpose of this study was exploratory, and it will be necessary to confirm the results of this work in a larger study. An additional issue related to the analysis of these data is that the mathematical model used to construct the dose–response curves treated the measured radiation fields as independent data points. This approach may have resulted in an overestimation of the statistical significance as some intra-individual association may have existed between the outcomes. To address this potential problem, an analysis was performed that restricted the observations to only the bolus-covered part of the photon field (Fig. 1). This field was chosen because it had the largest range in absorbed radiation dose and provided the highest number of responses (Table 1). Even with this limitation to just one field per patient, the dose–response curves for those with or without the 5557 G→A polymorphism remained significantly different from each other when analyzed by logistic regression (p<0.02) (Fig. 4). However, owing to the reduced number of observations and a smaller range in absorbed radiation dose, ED_{50} values and enhancement ratios with confidence intervals could not be determined by logit analysis.

It has previously been reported that both the incidence and severity of late normal tissue reactions after radiotherapy increase with time of follow-up (28). Although this might potentially constitute a problem, the mean follow-up time for carriers of the 5557 G>A SNP (1345 days) was nearly the same as for those patients who did not possess this variant (1399 days). Thus, the observed difference in fibrosis risk cannot be attributed to differences in length of follow-up.

Fig. 5. Dose–response curves for subcutaneous fibrosis in patients with either the G→A polymorphism at nucleotide 5557 or not possessing this alteration when the analysis was exclusively based on observations from the bolus-covered part of the photon field (i.e., one observation per patient).
Approximately 15–20% of the general population (31) possesses an adenosine in place of a guanine at nucleotide position 5557 in ATM resulting in substitution of aspartic acid to asparagine acid 1853 in the encoded protein. The results of this study are consistent with Angela et al. (11) who reported an association between possession of the 5557 G→A polymorphism and radiosensitivity, although the correlation found in that study was for patients homogamous for this polymorphism. In a recently published study, a non-significant overrepresentation of the ATM 5557 A allele was found among breast cancer patients with reduced survival in breast appearance after postlumpectomy radiotherapy (32). In addition, an association which did not achieve statistical significance owing to the small sample size, was reported between this SNP and late morbidity in prostate cancer patients (12).

Although there is now substantial evidence supportive of ATM as a gene associated with clinical radiosensitivity, it is nevertheless highly likely that this is not the only gene whose alteration is responsible for adverse radiotherapy response. Among the additional radiosensitivity candidate genes that have been identified as having an association with enhanced radiation responses are TGFBR1, XRCCI, XRCC8, SOD2, and AHR2. In a previously published study based on the same patient cohort used in the present investigation, it was observed that the risk of radiation-induced fibrosis was positively associated with the Pro/Pro genotype at codon 10 and the T/T genotype in position −509 of TGFBR1. In addition, the SOD2 codon 16 Val/Val, XRCC3 codon 241 Thr/Thr, and XRCCI codon 399 Arg/Arg genotypes were associated with enhanced radiosensitivity (29). Two separate studies examined polymorphic sites in TGFBR1 and also found an association between the −509 T/T and codon 10 Pro/Pro genotypes with the development of late normal tissue damage (32, 33). Another study screened three SNPs in XRCCI and detected an association with radiosensitivity for patients possessing either the codon 194 Arg/Thr alone or in combination with the codon 399 Arg/Thr genotype (34). It has also been reported that a T→C transition at position 1440 of the open reading frame of AHR2 was found in 6 of 19 radiation-sensitive cancer patients (35). An important distinction between the patient population reported upon in this paper, compared to those in other studies, is that the Danish patients were not selected for screening based upon the development of late effects. Generally, it is difficult to screen unselected populations as the incidence of late effects is too low to provide a sufficient number of cases to yield statistically significant results. Because many of the patients in this study were treated with high biologic doses, there was an adequate number of subjects who developed late effects without specifically selecting patients based upon their radiation response.

As described above, associations with risk of radiation-induced fibrosis have previously been detected for SNPs in the TGFBR1, SOD2, XRCCI, and XRCC3 genes within the 41 patients screened in the present study. Based on this observation, a model for estimation of fibrotic risk based on multiple SNPs was established. According to this model, the ED_{50} values for Grade 3 fibrosis correlated with the total number of “risk alleles” harbored at nine polymorphic sites in these genes (29). Considering the current indications that the ATM 5557 G→A (codon 1883 Asp/Asn) polymorphism may also influence risk of radiation-induced fibrosis, we incorporated this SNP in a similar analysis of multiple SNPs. In the original model (29), three TGFBR1 polymorphisms (position −509, codon 10, and codon 25) were included. However, due to the existence of tight genetic linkage between these SNPs, they aggregate into a limited number of well-defined haplotypes (6). Therefore, these three SNPs should probably not be regarded as independent risk factors. Furthermore, recent in vitro data have suggested a functional impact of the codon 10 SNP on the secretion rate of transforming growth factor beta 1 (TGFβ1) (36). Consequently, the analysis was restricted to this TGFBR1 SNP in the current model. Thus, the Asn, Arg, Thr, Ala, and Pro alleles at ATM codon 1853, XRCCI codon 399, XRCC8 codon 241, SOD2 codon 16, and TGFBR1 codon 10, respectively, were defined as putative “risk alleles.” The patients were grouped according to the total number of “risk alleles” they possessed. ED_{50} values were calculated for patients with 2, 3, 4, 5, and 6–7 “risk alleles” (8). The patients were grouped in this way to achieve approximately the same number of subjects in each group. Because the patients segregated differently with respect to the number of “risk alleles” harbored, this new model could not be directly compared with the original version. However, this analysis supports the hypothesis that clinical normal tissue radiosensitivity is determined by the combined influence of multiple genetic alterations (37). Furthermore, it is noteworthy that the model identified a subset of patients characterized by a high degree of radioresistance. Nonetheless, it should be stressed that this analysis was based on a limited number of subjects and that confirmation in independent studies is needed before reaching definitive conclusions concerning a possible subpopulation of radiation-resistant patients.
CONCLUSIONS

Based upon the results of this study, a hypothesis can be formulated, which will be tested in a larger cohort of patients, that the ATM 5537 G>A polymorphism, resulting in the codon 1853 Asn/Asp and Asn/Asn genotypes, is associated with the development of Grade 3 stromal fibrosis in breast cancer patients after postmastectomy radiation treatment.

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ABSTRACT- Purpose/Objective: The purpose of this study was to identify ATM gene sequence variants found specifically among African-American women that may predict for the development of adverse effects resulting from radiation therapy for breast cancer.

Methods: 34 African-American women and 73 non-African American women were screened for DNA sequence variations in the 62 coding exons of the ATM gene using DHPLC. All patients underwent breast conserving surgery and standard adjuvant radiation therapy for either DCIS or early stage breast cancer and had a minimum of two years of follow up. Chi-square and Fisher exact tests were used to compare groups.

Results: 53% (18/34) of the African-American and 22% (16/73) of the non-African-American patients were found to harbor ATM gene sequence alterations located within exons, or in short intronic regions flanking each exon that encompass putative splice sites (p=0.003). Furthermore, 26% (9/34) of the African-American versus 3% (2/73) of the non-African-American subjects possessed multiple ATM sequence alterations (p<0.001). Among African-American patients with ATM sequence variants, 72% (13/18) demonstrated a late radiation-induced adverse response. In contrast, 50% (8/16) of the African-American patients with no ATM sequence variation, manifested a late response (p=0.29). Among non-African-Americans, 81% (13/16) of those subjects with sequence variants exhibited late responses while only 51% (29/57) without sequence alternations, developed late effects (p=0.04). Of the 24 different variants identified, only 3 were shared between the two groups. Conclusions: We found a higher incidence of ATM gene variants in African American women. The variety and frequency of these polymorphisms appear to be unique to this population. In addition, African-American women had a higher incidence of multiple ATM variants compared to the non-African-American population. Whereas possession of ATM gene variants was predictive for late adverse responses to radiotherapy among non-African Americans, this finding did not reach statistical significance in the African American population, perhaps secondary to the small sample size. This research was supported by the Dept. of the Army grants DAMD 17-02-1-0502 and DAMD 17-02-1-0503.

Key words: ATM gene, African-American, Adverse radiotherapy effects, Breast Cancer
ATM Sequence Variants as Predictors for Late Normal Tissue Responses in Breast Cancer Patients Treated with Radiotherapy

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Purpose/Objective: To examine whether the presence of sequence variants in the ATM gene is predictive for the development of late radiation-induced adverse effects resulting from external beam radiation therapy for breast cancer.

Materials/Methods: 107 patients with a minimum of a 2-year follow-up underwent breast-conserving surgery and standard adjuvant radiation therapy for either DCIS or early stage breast cancer at three tertiary referral centers in the United States between 1990 to 2003. These patients were screened for DNA sequence variations in all 62 coding exons of the ATM gene. DNA was isolated from blood lymphocytes and each coding exon amplified using PCR. Genetic variants were identified using denaturing high performance liquid chromatography (DHPLC). The clinical course of each genetically characterized patient was obtained from a database of patients treated and examined during follow-up visits. The RTOG/EORTC late morbidity scoring schemes for skin and subcutaneous normal tissues were applied to quantify radiation-induced effects. The chi-square test was used to compare groups with respect to categorical endpoints (e.g. radiation-induced late effects).

Results: 34 of the 107 screened patients were found to carry ATM sequence alterations located within exons, or in short intronic regions flanking each exon that encompass putative splice sites. For this group, 77% (26/34) exhibited at least one form of adverse response. In contrast, of the 73 patients who did not harbor an ATM sequence variation, 51% (37/73) manifested radiation-induced adverse responses (p=0.02). Nine of the patients in this study specifically possessed the G→A transition polymorphism at nucleotide 5557, which results in substitution of asparagine for aspartic acid at position 1853 of the ATM protein. For this group, 100% (9/9) exhibited an adverse response. In contrast, of the 98 patients who did not have this polymorphism, 55% (54/98) manifested a late response (p=0.02).

Conclusions: Possession of sequence variants in the ATM gene is predictive for the development of late adverse radiotherapy responses among breast cancer patients treated with adjuvant radiation therapy. In particular, the 5557 G→A polymorphism is associated with the development of adverse late responses. In addition, the number of patients without ATM sequence variants who nevertheless developed late normal tissue effects suggests that genetic variants in radiation response genes other than ATM may also play a role conferring radiosensitivity, and could therefore serve as additional predictors of adverse radiation effects.

Acknowledgement: This research was supported by Department of the Army grants DAMD 17-02-1-0502 and DAMD 17-02-1-0503.
Impact of Low Dose Rate Prostate Brachytherapy on the Sexual Health of Men with Normal Pre-treatment Sexual Function; an Analysis at Seven-years Minimum Follow-up

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Purpose/Objective: To evaluate the impact of prostate brachytherapy on the sexual health of men with at least seven years of prospective evaluation and normal pre-treatment erectile function (EF).

Materials/Methods: 223 patients with T1b to T3a prostate cancer and median age of 66 years (range: 50 – 82 were treated with permanent seed implantation from 11/1990 to 3/1998 and followed from 7 to 14.1 years (median 8.2) using prospective quality of life measures. Pre-treatment parameters were as follows: PSA (range: 1.7 – 300, median 8.5), stage ( ≠ T2a in 63%, ≥ T2b in 37%), Gleason score ( ≤ 6 in 77%, 7 in 15% and 8 – 10 in 8%). Patients were treated with implant alone (125I or 103Pd) in 53%, hormonal therapy and implant in 38%, and implant and external beam (± hormonal therapy) in 9%. 28 men were between 50 – 59 years old at implant, 117 between 60 – 69, 77 between 70 – 79 and 1 between 80 – 82 years old. EF was assessed using a physician-assigned potency rating ranging from 0 to 3 (0= no erections, 1= ability to have erections but insufficient for vaginal penetration, 2= erectile function sufficient for vaginal penetration but suboptimal, 3= normal erectile function). Beginning in June 2000, the validated International Index of Erectile Function-5 (IIEF-5) was used as a complimentary method to quantify late EF. No adjustment was made to differentiate sexual function with or without an EF pharmacological intervention. The Pearson’s chi square test and Student t-test were used to compare groups.

Results: 131/223 (59%) had normal erectile function (EF=3) prior to their brachytherapy procedure. Of these men, 51/131 (40%) were using either a phosphodiesterase type 5 inhibitor 44/51 (86%), yohimbine 2/51 (4%) or alprostadil 5/51 (10%) at last follow-up evaluation. Age at implant was highly predictive of current EF. 23/25 (92%) of patients age 50 – 59 had a current EF≠2. Patients age 60 – 69yo and 70 – 78yo had an EF≠2 in 48/75 (64%) and 18/31 (58%) of individuals (p=0.01). Current IIEF-5 ≥16 also correlated highly with age: 50 – 59 yo 16/25 (64%), 60 – 69 yo 20/75 (27%), 70 – 78 yo 6/31 (19%) (p=0.0005). The incidence of diabetes, hypertension, smoking and use of adjuvant hormone therapy were evenly distributed among age groups.

Conclusions: At seven years minimum follow-up a significant percentage of men with normal pre-treatment sexual function were able to experience a high rate of erectile function as quantified by the IIEF-5 and physician assigned scoring system. For patient’s less than 60 years old with good erectile function prostate brachytherapy appears to confer a very high probability of long-term erectile function.
Assessment of Post-Brachytherapy Sexual Function: A Comparison of the IIEF-5 and the MSEFS

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Purpose/Objective: Erectile dysfunction (ED) remains an undesirable side effect in many men following treatment for prostate cancer. To overcome physician bias in assessment of potency following treatment, patient-assessed validated questionnaires were developed. The Mount Sinai Erectile Function Score (MSEFS) (a physician-assigned potency rating) was developed for our brachytherapy program starting in 1990 (J. Urol., 165: 436–439, 2001). In 2000, patients were asked to independently fill out the International Index of Erectile Function-5 (IIEF-5), also known as the Sexual Health Inventory for Men (SHIM), as part of their evaluation and follow-up. This study compares the two methods of assessment and describes potency following brachytherapy.

Materials/Methods: Between 1990 and 2004, 1,202 patients with T1,T2, or T3 prostate cancer were treated with ultrasound-guided radioactive seed implantation with or without external beam irradiation and had at least one visit where both MSEFS and IIEF-5 assessment were completed. At each of the 3,161 visits, patients were assigned a MSEFS ranging from 0 to 3 (0-no erections, 1-ability to have erections but insufficient for vaginal penetration, 2-erectile function sufficient for vaginal penetration but suboptimal, 3-normal erectile function) and completed an IIEF-5 with a possible maximum total score of 25 (severe ED 1–7), moderate ED 8–11, mild to moderate ED 12–16, mild ED 17–21, no ED 22–25. Correlations were performed using the Spearman rho test. Follow-up visits were done at 6-month intervals, ranging from none to 165 months, median 36 months.

Results: The MSEFS significantly correlated with the total IIEF-5 scores on all comparisons with p values <0.001. The coefficient was 0.65 for comparisons done on the initial consultation date and 0.76 for all visits. On subsequent follow up visits, the correlations remained strong. The correlation coefficients for follow-up visits 1 through 10 were: 0.76, 0.74, 0.74, 0.78, 0.77, 0.78, 0.79, 0.78, 0.92 and 0.87, respectively. 116 patients were assigned to be potent (MSEFS of 2 or 3) before brachytherapy. Of the 116, we have follow-up on 78; 53 of these patients (68%) remained potent as defined by a MSEFS score of 2 or 3 at last visit. The corresponding last IIEF-5 scores for these patients were: 1–7 in 33%, 8–11 in 9%, 12–16 in 23%, 17–21 in 21% and 22–25 in 14%.

Conclusions: Our physician-assigned potency scale correlates well with the IIEF-5. Because the IIEF-5 is weighted considerably toward a patient’s degree of sexual desire, it cannot fully replace, the physician scale in assessing the development of ED after radiation. Furthermore, more insight into patient’s erectile function after brachytherapy may be gotten if the IIEF-15, from which the IIEF-5 was developed, is used instead of the IIEF-5, in conjunction with our MSEFS.
ATM SEQUENCE VARIANTS ARE PREDICTIVE OF THE DEVELOPMENT OF ERECTILE DYSFUNCTION AMONG PATIENTS TREATED FOR PROSTATE CANCER WITH 125IODINE

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Purpose: To examine whether the presence of sequence variants in the ATM (mutated in ataxia telangiectasia) gene is predictive for the development of radiation-induced erectile dysfunction resulting from 125I prostate brachytherapy for early stage prostate cancer.

Materials and Methods: 37 patients, with a minimum of one-year follow-up, who underwent 125I prostate brachytherapy of early stage prostate cancer were screened for DNA sequence variations in all 62 coding exons of the ATM gene using denaturing high performance liquid chromatography (DHPLC). The clinical course of their erectile function for each genetically characterized patient was obtained from a database of 2220 patients implanted at Mount Sinai Hospital since 1990.

Results: 21 ATM sequence alterations located within exons, or in short intronic regions flanking each exon, were found in 16 of the 37 patients screened. Nine of the patients with sequence alterations specifically possessed missense mutations, which encode for amino acid substitutions, and are therefore more likely to possess functional importance. Of those patients with missense mutations who were potent prior to brachytherapy, 5/8 (63%) developed prospectively evaluated erectile dysfunction (ED) as opposed to 2/20 (10%) without these sequence alterations (p=0.009). Severe ED as quantified by IIEF-5 occurred in 5/9 (56%) patients with missense mutations compared to 3/27 (12%) of patients without these sequence abnormalities (p=0.01).

Conclusion: Possession of sequence variants in the ATM gene, particularly those that encode for an amino acid substitution, is predictive for the development of erectile dysfunction among patients treated with 125I prostate brachytherapy.

Key Words: ATM gene, Radiation sensitivity, DHPLC, Prostate cancer, Brachytherapy, Erectile Dysfunction.
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**GRANTS**

Sponsor: National Institute of Health Loan Repayment Program  
Principle Investigator: Cesaretti JA  
Project entitled,”ATM Heterozygosity and the Development of Radiation-Induced Erectile Dysfunction and Urinary Morbidity Following Radiotherapy for Prostate Cancer.” (7/1/05-6/30/07)

Sponsor: American Cancer Society  
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Prostate Cancer Research Program.  
Sponsor: Department of Defense.  
Principle Investigator: Cesaretti JA (60% effort)  
Mentors: Stock RG, Rosenstein BA  
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(7/1/04-6/30/09)

**PUBLICATIONS**


**ABSTRACTS**


Kollmeier MA, Stone NN, **Cesaretti JA**, Stock RG. “Comparison of Race and Prostate Cancer Outcome in Patients Treated with Brachytherapy.” *Brachytherapy* 2004 May 1; 3(1): 290.

**PRESENTATIONS**


**Cesaretti JA.** “Interactive Ultrasound Guided Prostate Brachytherapy; The Mount Sinai Experience.” First Annual Radiation Oncology Symposium, Galliera Hospital, November 2003, Genoa, Italy.

**Cesaretti JA.** “Real Time Brachytherapy: The American Experience.” International Course on Brachytherapy, San Paolo Hospital, February 2004, Savona, Italy.


POSTER DISCUSSION


POSTERS

