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CLINICAL INVESTIGATION **Normal Tissues**

ATM SEQUENCE VARIANTS ARE PREDICTIVE OF ADVERSE RADIOTHERAPY RESPONSE AMONG PATIENTS TREATED FOR PROSTATE CANCER

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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 adverse responses resulting from ^{125}I prostate brachytherapy for early-stage prostate cancer.

Materials and Methods: Thirty-seven patients with a minimum of 1-year follow-up who underwent ^{125}I 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. The clinical course and postimplant dosimetry for each genetically characterized patient were obtained from a database of 2,020 patients implanted at Mount Sinai Hospital after 1990.

Results: Twenty-one *ATM* sequence alterations located within exons, or in short intronic regions flanking each exon, were found in 16 of the 37 patients screened. For this group, 10 of 16 (63%) exhibited at least one form of adverse response. In contrast, of the 21 patients who did not harbor an *ATM* sequence variation, only 3 of 21 (14%) manifested radiation-induced adverse responses ($p = 0.005$). 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. For this group, 7 of 9 (78%) exhibited at least one form of adverse response. In contrast, of the 28 patients who did not have a missense alteration, only 6 of 28 (21%) manifested any form of adverse response to the radiotherapy ($p = 0.004$). Of the patients with missense variants, 5 of 9 (56%) exhibited late rectal bleeding vs. 1 of 28 (4%) without such alterations ($p = 0.002$). Of those patients who were at risk for developing erectile dysfunction, 5 of 8 (63%) patients with missense mutations developed prospectively evaluated erectile dysfunction as opposed to 2 of 20 (10%) without these sequence alterations ($p = 0.009$).

Conclusions : Possession of sequence variants in the *ATM* gene, particularly those that encode for an amino acid substitution, is predictive for the development of adverse radiotherapy responses among patients treated with ^{125}I prostate brachytherapy. © 2005 Elsevier Inc.

ATM gene, Radiation sensitivity, DHPLC, Prostate cancer, Brachytherapy.

INTRODUCTION

Ataxia-telangiectasia (A-T) is a rare autosomal recessive genetic syndrome caused by genetic mutations in both copies of the *ATM* gene (1). Generally, these mutations result in truncation of the encoded protein (2). A-T is characterized clinically by cerebellar degeneration, ocular telangiectasias, and immunodeficiency. Of particular interest has been the observation that radiotherapy patients with A-T experience devastating side effects after exposure to ionizing radiation (3), including severe skin necrosis and organ dysfunction. Understanding the function of the protein encoded by *ATM* advanced greatly after cloning of the *ATM* gene. Subsequent elucidation of the activity of the ATM protein revealed a central role orchestrating the cellular response to DNA double-strand breaks (4, 5). ATM-dependent modifications of the proteins encoded by the *p53*, *BRCA1*, *CHK2*, *NBS1*, *FANCD2*, *CDC25A*, and *RAD17* genes modulate cell cycle progression and DNA repair in response to environmental assaults and ionizing radiation (6–18).

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Table 1. Patient characteristics in addition to baseline urinary, rectal, and erectile function

Characteristic	Number of patients (%)
Median age	63 years (range: 48–78 years)
Coronary artery disease	12 (32)
Angioplasty	4 (11)
Hypertension	6 (16)
Coronary bypass surgery	3 (8)
Myocardial infarction	2 (5)
Not otherwise specified	1 (3)
Active smoker	4 (11)
Reformed smoker	9 (24)
Diabetes	3 (8)
Pretreatment American Urologic Association urinary function score	
Good (0–7)	28 (76)
Moderate (8–19)	7 (19)
Severe (20–35)	2 (5)
History of transurethral prostate resection before implant	1 (3)
Preimplant ultrasound prostate volume	
≤35 cm^3	8 (22)
36–50 cm^3	20 (54)
>50	9 (24)
Erectile function	
3 - Optimal	22 (60)
2 - Suboptimal but sufficient	6 (16)
1 - Insufficient	5 (14)
0 - None	4 (11)
Ulcerative colitis/Crohn disease	1 (3)
Hemorrhoids	7 (19)

Although the occurrence of alterations in both copies of the *ATM* gene is rare, individuals who are heterozygous carriers of a single *ATM* mutation may constitute more than 1% of the general population. It has been shown that cells derived from heterozygous individuals exhibit an intermediate degree of radiosensitivity between those of wild-type and homozygously mutated cells derived from people with A-T (19–21). Animal studies have found that heterozygous $ATM^{+/-}$ mice are more susceptible to radiation-induced cataracts compared with wild-type $ATM^{+/+}$ counterparts (22). These discoveries have led to the hypothesis that possession of one altered copy of the *ATM* gene may predispose patients receiving radiotherapy to adverse reactions associated with this treatment.

Several studies have screened the *ATM* gene in patients who displayed clinically abnormal radiosensitivity. Initially, the results of these studies were negative, primarily because the samples were analyzed using a test for protein truncation (23, 24). However, it is now recognized that the most prevalent *ATM* sequence alterations detected specifically in cancer patients are missense mutations causing amino acid substitution in the encoded protein (2). In view of this understanding, further studies were conducted using assays designed to detect this class of genetic alterations, and several positive findings correlating clinical radiosensitivity and *ATM* mutations have since been reported (21, 25, 26).

Table 2. Clinical tumor characteristics

Characteristic	Number of patients (%)
PSA (ng/mL)	(range: 1.2–15, median: 6)
≤4	3 (8)
>4–10	31 (84)
>10–20	3 (8)
Gleason score	
5	5 (14)
6	31 (84)
7	1 (3)
Stage (AJCC 2002)	
T1c	25 (68)
T2a	8 (22)
T2b	4 (11)

One study, screening the *ATM* gene of 46 breast cancer patients treated with radiotherapy, revealed that 3 of 4 patients possessing an *ATM* missense mutation developed Grade 3–4 skin fibrosis. In contrast, none of the patients without a missense mutation developed this type of adverse radiotherapy response (26). Another study with a more limited genetic analysis of the *ATM* gene in which only 8 specific variants were genotyped reported that 4 of 6 breast cancer patients homozygous for the G→A transition polymorphism at nucleotide 5557, which transforms an aspartic acid into an asparagine at position 1853 of the protein, exhibited clinically abnormal radiosensitivity (25). In addition, it was reported that a patient discovered to be heterozygous for insertion of a guanine at position 3637, resulting in a frame-shift leading to a stop codon (TAG) at nucleotide 3681, experienced severe skin and subcutaneous tissue effects after conventional radiation therapy in the adjuvant setting for breast cancer (21). Cells from this patient displayed a radiosensitivity between the values for normal cells and those from patients with AT. Finally, Hall *et al*. reported that 3 of 17 prostate cancer patients exhibiting radiation-related morbidity after radiotherapy possessed *ATM* mutations (27).

The purpose of this study was to examine the hypothesis that the presence of *ATM* sequence alterations is predictive for the development of adverse radiotherapy responses among prostate cancer patients. We have screened the expressed portions of *ATM* and short adjacent intronic regions that may encompass putative splice sites for DNA sequence variations (28). This work was accomplished using denaturing high-performance liquid chromatography (DHPLC) with DNA samples derived from lymphocytes obtained from an unselected group of 37 men treated with low-dose-rate ^{125}I brachytherapy for prostate cancer. We explore any potential association of acute and late erectile, rectal, and urinary functional outcomes with *ATM* alterations using standard morbidity measuring tools.

METHODS AND MATERIALS

Patients

Peripheral blood lymphocytes were collected from a consecutive series of 37 patients seen for periodic evaluation who under-

Table 3. The postimplant dosimetric parameters of all patients

Implant characteristics	Median (range)
Total activity (mCi)	42 (27.3–62.6)
Needle number	24 (16–29)
Seed number	103 (70–171)
Dose to 90% of the prostate (Gy)	196 (156–220)
Dose to 100% of the prostate (Gy)	111 (78–139)
Volume of prostate receiving 150% of prescription dose (%)	68 (36–84.3)
Dose to 30% of the urethra (Gy)	228 (23–265)
Amount of rectum receiving 100% prescription dose (cm^3)	0.7 (0.01–3.56)

went ^{125}I prostate brachytherapy for early-stage prostate cancer between June 1997 and April 2002. All patients had biopsy-proven adenocarcinoma with central pathology review performed on all specimens. Patients were staged according to American Joint Cancer Commission standard (29). Patient and tumor characteristics are outlined in Tables 1 and 2. Brachytherapy was administered via the transperineal approach using a transrectal ultrasound probe to direct the placement of each radioactive source within the prostate (30). The implant characteristics are enumerated in Table 3. The prescription dose for all implants was 160 Gy corrected for TG-43 recommendations (31). Patients returned at approximately 4 weeks after the implant for detailed CT-based dosimetric analysis. In this study, a comprehensive dose–volume histogram analysis was available for the bladder, rectum, urethra, and prostate of each patient. Patient follow-up included digital rectal examinations and serial PSA measurements. Biochemical failure was defined using the American Society for Therapeutic Radiation and Oncology consensus definition (32).

Definition of adverse response

Patient clinical data were available from the departmental prostate cancer tissue repository database, which prospectively collected data for the 2,020 patients who underwent prostate brachytherapy at Mount Sinai between June 1990 and February 2004. All patients underwent a detailed history and physical examination before implantation followed by a directed history and physical examination at 6-month-interval follow-up evaluations. Acute and late rectal toxicities were graded using the Radiation Therapy Oncology Group (RTOG) morbidity criteria (33). Patients who developed either RTOG grade level 1 or 2 rectal effects were classified as having an adverse response. Urinary tract morbidity was prospectively measured using the American Urologic Association Symptom Score (AUASS) sheet that was administered before the implant and at each follow-up evaluation (34). The urinary quality of life score from the AUASS was used for analysis with a score of 6 or "terrible" long-term urinary quality of life classified as an adverse response. Erectile function was assessed using the following scoring system: 0, complete inability to have erections; 1, able to have erections but insufficient for intercourse; 2, can have erections sufficient for intercourse but considered suboptimal; and 3, normal erectile function. The derivation and relevance of this scoring system have been previously described (35, 36). For this analysis, a decline by 2 points was considered a significant prospective decline in erection function, and these patients were classified as having an adverse response. In addition, beginning in June 2000, the validated International Index of Erectile Function (IIEF-5) was used as a complementary method to better quantify late erectile dysfunction (ED) (37). A score of 0–2 was judged as an adverse response. The last completed form was used for this study, because the relatively recent development of the IIEF-5 did not allow for a prospective evaluation in most patients.

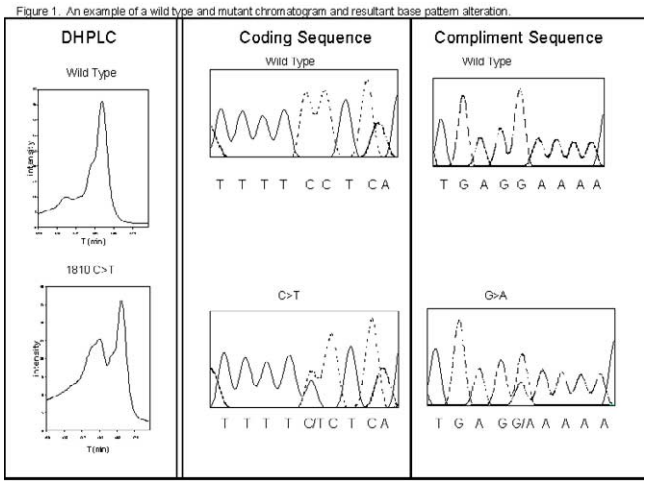

Fig. 1. An example of a wild-type and mutant chromatogram and resultant base pattern alteration.

The goals of the project were discussed with each patient as outlined by the guidelines approved in the institutional review board protocol, and written informed consent was obtained.

ATM *exon characterization*

DNA isolation from lymphocytes was accomplished using Ficoll separation as described previously (38). Polymerase chain reaction (PCR) 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 (39). DHPLC analysis was performed on a WAVE Nucleic Acid Fragment Analysis System (Transgenomic, Omaha, NE) using buffer gradient and temperature conditions calculated using WAVEmaker software (version 3.3; Transgenomic) designed for this purpose. An example of a wild-type and mutant chromatogram and resultant base pattern alteration is seen in Fig. 1. Exons with an aberrant DHPLC chromatogram underwent DNA forward and reverse sequencing using an ABI PRISM 377 DNA Sequencer (Foster City, CA).

Statistical analysis

Analyses were performed using the Statistical Package for Social Sciences (SPSS, Chicago, IL) software. Differences in proportions were derived using the Fisher's exact *t*-test. A two-sided p value of ≤ 0.05 was considered to indicate statistical significance.

RESULTS

A total of 21 *ATM* sequence variants, representing 17 different alterations, were detected in expressed portions of the gene, or within 10 nucleotides of each exon encompassing potential splice sites, in 16 of the 37 patients screened (Table 4). It should be noted that most of the sequence variants detected in this group of patients represent genetic

Table 4. Each patient with toxicity, genetic, comorbid, and follow-up data

Patient (#)	*ATM* alteration	Prospective erectile decline	Last follow-up IIEF-5	Rectal bleeding	Urinary quality of life	D_{90} ‡ (Gy)	Comorbidities	Follow-up (months)
1	4473C>T, 149.1F>F	No	24	No	1	184	CAD	21
2		No	18	No	4	192		36
3	4578C>T, 1526P>P; 5557G>A, 1853D>N	Yes	2	RTOG 1	6	180		67
4		No	20	No	3	208	Tob	37
5		No	16	No	2	205	Tob	29
6		No	24	No	1	165		36
7		*	10	No	0	191		70
8		No	†	No	2	220		49
9	1810C>T, 604P>S	Yes	16	No	6	208		19
10	378T>A, 126D>E; IVS7-8insT; 1176C>G, 392G>G	Yes	1	No	2	197	DM	12
11	2685A>G, 895L>L; 2614C>T, 872P>S	Yes	1	RTOG 1	1	205		40
12	IVS38-8T > C	No	24	No	1	159		60
13		*	23	No	2	174	DM, CAD	31
14		No	1	No	3	210	CAD	20
15	IVS38-8T>C	No	19	No	4	164	Tob	39
16		No	14	No	0	183		59
17		*	5	No	0	169		44
18		No	22	No	2	220		40
19		No	12	No	2	206		26
20		*	21	No	2	199	Tob	37
21		*	2	No	2	174	DM, CAD	25
22	198A>C, 66K>K	*	1	No	1	217		40
23		No	23	No	1	160		25
24		Yes	9	No	2	184		39
25		*	6	No	4	218		32
26	4388T>G, 1463F>C; 1810C>T, 604P>S	*	2	RTOG 2	2	209	CAD	13
27		No	15	No	4	205		32
28	5071A>C, 1691S>R	Yes	1	RTOG 2	2	192		45
29	3161C>G, 1054P>R	No	19	No	2	197		27
30	IVS62+8A>C	No	19	RTOG 1	0	217	CAD	47
31	4578C>T, 1526P>P	Yes	8	No	0	193		26
32	2038T>C, 680F>L	No	19	RTOG 1	0	219		31
33		No	24	No	2	162		71
34		*	3	No	0	168	CAD	69
35	5557G>A, 1853D>N	No	20	No	0	186		58
36		No	18	No	1	197		43
37	IVS22-6T>G	No	22	No	3	210		29

Abbreviations: CAD = coronary artery disease; DM = diabetes mellitus; RTOG = Radiation Therapy Oncology Group; Tob = active smoker.

* Patient had a suboptimal erectile function before implant.

† Patient did not fill out IIEF-5.

‡ Dose to 90% of the prostate gland via brachytherapy.

alterations that have been previously reported as polymorphisms in *ATM* (40–42). For this group, 10 of 16 (63%) exhibited at least one form of adverse radiotherapy response. In contrast, of the 21 patients who did not harbor an *ATM* sequence variation, only 3 of 21 (14%) manifested any form of adverse response ($p = 0.005$). There were 9 patients found carrying missense mutations encoding for amino acid substitutions in the *ATM* protein. Missense mutations represent sequence alterations that are more likely to impact functional integrity. Of the 9 patients with missense mutations, 7 (78%) exhibited at least one form of adverse response. In contrast, of the 28 patients who did not have a missense mutation, only 6 of 28 (21%) manifested any form of adverse response to the radiotherapy ($p = 0.004$). Moreover, 5 of 9 (56%) patients with missense mutations exhibited an adverse response in two or three of the three organ systems evaluated (Patients 3, 9, 11, 26, and 28), whereas none of the remaining 28 patients without such sequence changes exhibited morbidity in more than one evaluated organ system ($p = 0.003$).

RTOG Grade 1 or 2 rectal bleeding was seen in 5 of 9 (56%) patients with missense mutations vs. 1 of 28 (4%) of

Table 5. Univariate analysis of variables that may predict for urinary, erectile, and rectal morbidity. All p values derived from 2-sided Fisher's exact t-test

Variable	Two radiation morbidities	SHIM erectile decline	Prospective erectile decline	Rectal Bleeding RTOG 1,2	Urinary quality of life "terrible"
Dose ≥210 Gy	1	0.34	0.29	0.14	1
Diabetes	1	0.12	0.25	1	1
Smoking	1	0.56	0.55	1	1
Coronary artery disease	1	0.17	0.55	0.32	1
ATM alteration	0.0003	0.01	0.009	0.002	0.05

Abbreviations: RTOG = Radiation Therapy Oncology Group; SHIM = Sexual Health Inventory for Men.

those without these genetic alterations ($p = 0.002$). The median amount of rectal tissue exposed to the prescription dose of 160 Gy among the individuals with rectal bleeding was 0.87 cm^3 (range, 0.04–1.24), which is below previously published rectal dosing parameters for prostate brachytherapy and predicts a low probability of late radiation-induced proctitis based upon dose alone (43).

Severe ED as quantified by IIEF-5 occurred in 5 of 9 (56%) patients with missense mutations compared with 3 of 27 (12%) of patients without these sequence abnormalities ($p = 0.01$). When considering only patients with sufficient erectile function before radiotherapy prospectively, a significant correlation was also noted between the development of erectile dysfunction in men with missense mutations, 5 of 8 (63%), as opposed to 2 of 20 (10%) in men without these types of variants ($p = 0.009$). In addition, both patients who reported a "terrible" urinary quality of life had *ATM* missense alterations (2 of 9, 22%) vs. 0 of 28 patients without missense alterations ($p = 0.05$).

The effects of total dose, diabetes, coronary artery disease, and active tobacco use were analyzed separately in relation to each of the adverse responses defined. No independent variable achieved statistical significance (Table 5), other than the presence of an *ATM* sequence alteration. In addition, none of the patients experienced a palpable local or biochemical disease recurrence.

DISCUSSION

Sixty-three percent (10 of 16) of prostate cancer patients treated with ^{125}I brachytherapy who were found to be carriers of sequence variants either within the exons or in short intronic regions flanking exons of the *ATM* gene developed at least one form of urinary, sexual, or rectal adverse response. In contrast, only 14% (3 of 21) of patients without *ATM* sequence variations displayed some form of adverse response. Furthermore, when only those patients specifically harboring missense mutations are considered, 78% of these patients developed adverse responses compared with 21% who did not possess these types of sequence abnormalities. The results of this study are supportive of the hypothesis that genetic alterations in the *ATM* gene are predictive for the development of adverse responses resulting from radiotherapy.

Radiation-induced permanent sexual dysfunction has a substantial negative impact on the quality of life of men treated for prostate cancer. Brachytherapy series have reported a widely variable incidence of reduced sexual potency after implantation (35, 36, 44–48), ranging from 14% to 50%. In this unselected series, 30% (11 of 37) of patients overall had erectile dysfunction, a figure that is consistent with previous reports. Of even greater significance, however, is that 63% of patients in this study with good preirradiation erectile function developed prospectively evaluated ED if they possessed an *ATM* missense mutation vs. 10% of men without such an alteration. The correlation of ED with *ATM* missense mutations was also apparent when men were evaluated only at last follow-up with the validated IIEF-5. Using this evaluation tool, it was found that 56% of patients with missense mutations, vs. 12% without these genetic changes, developed severe ED. These findings attest to the predictive power of *ATM* mutational status for ED and warrant validation of this striking correlation in a larger group of individuals.

A second significant correlation observed in this study is that of postradiation rectal bleeding with *ATM* sequence alterations. All of the patients who experienced late rectal bleeding had *ATM* sequence alterations. The 2 patients who manifested comparatively severe rectal bleeding, RTOG Grade 2, had DNA missense mutations. In particular, the patient with the most serious rectal bleeding was a carrier of two nonconservative missense mutations and displayed this morbidity at only 5 months after radioactive seed implantation, rather than the more typical 1.5 to 2 years. This patient underwent colonoscopy and biopsy, which identified distal proctitis and an absence of the classic telangiectasias. Patients who undergo brachytherapy receive relatively low rectal doses compared with the use of external beam irradiation involving a larger pelvic field. Most radiation-related rectal bleeding secondary to prostate cancer radiotherapy is self-limited and innocuous, but there are patients who are inordinately affected and develop rectourethral fistulas (49, 50). In these instances, it could prove even more

important to predict which patients may be radiosensitive.

With respect to the correlation of urinary symptoms with *ATM* abnormalities, the 2 patients reporting a late "terrible" urinary quality of life at last follow-up both had nonconservative missense mutations. The spectrum of affected organs for these patients included a severe decline in prospectively measured erectile function. In addition, 1 of the 2 patients had rectal bleeding. The AUASS form appears effective in quantifying the most severe urinary morbidity, but there is a relatively long symptomatic period after the implant that may decrease this instrument's power to discern differences in intermediate-term urinary function.

It may be anticipated that the tumors possessed by patients harboring ATM mutations could also be radiosensitive and that these men may exhibit higher levels of tumor control compared with patients not harboring sequence alterations. However, the patients included in this study had low-risk prostate cancer, and all were treated with optimal implants based upon evaluation of their postbrachytherapy dosimetric studies (51). It is therefore not surprising that none of the patients screened in this study failed treatment. As reported previously by our institution, these patients have an expected freedom from PSA failure of 94% at 8 years (52). Therefore, it was not possible to examine whether *ATM* genetic status conferred tumor radiosensitivity.

Clearly, there is a strong association between sequence variants in the *ATM* gene and increased clinical radiosensitivity. Nevertheless, it is highly probable that *ATM* is not the only gene whose alteration can predispose patients to adverse radiotherapy responses. Thus, the patients in this series who exhibited pronounced radiation-related morbidity, but proved negative for *ATM* sequence variants, may possess alterations in other genes associated with radiation response. Among the additional radiosensitivity candidate genes that have now been linked with enhanced radiation effects are *TGFβ1*, *XRCC1*, *XRCC3*, *SOD2*, and *hHR21* (53–56). Alterations in these genes are also likely to serve as important potential predictors of adverse radiotherapy response. In view of the clinical associations observed between radiation sensitivity and the *ATM* gene in this study, combined with the reported association of other genes, it is critical that comprehensive genetic screening of radiotherapy patients for DNA sequence variations in candidate genes associated with radiation response be accomplished, because the results of such studies could yield significant patient benefit.

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