Int. J. Radiation Oncology Biol. Phys., Vol. 64, No. 3, pp. 776–783, 2006
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0360-3016/06/$–see front matter

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doi:10.1016/j.ijrobp.2005.09.014

CLINICAL INVESTIGATION **Normal Tissues**

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

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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 generate dose–response curves and to estimate the dose that resulted in a 50% incidence of Grade 3 fibrosis (ED_{50}).

Results: A total of 26 genetic alterations in the expressed portions of the *ATM* gene, or within 10 bases of each exon in regions encompassing putative splice sites, were detected in 22 patients. The ED_{50} (95% confidence interval) of 60.2 (55.7–65.1) Gy calculated for patients without a sequence variation did not differ significantly from the ED_{50} of 58.4 (54.0–63.1) Gy for the group of patients with any *ATM* sequence abnormality. The ED_{50} of 53.7 (50.2–57.5) Gy for those patients who were either homozygous or heterozygous for the G→A polymorphism at nucleotide 5557, which results in substitution of asparagine for aspartic acid at position 1853 of the ATM protein, was substantially lower than the ED_{50} of 60.8 (57.0–64.8) Gy for patients not carriers of this sequence alteration. This resulted in an enhancement ratio (ratio of the ED_{50} values) of 1.13 (1.05–1.22), which was significantly greater than unity.

Conclusion: 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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***ATM*, Breast cancer, DHPLC, Fibrosis, Radiation sensitivity.**

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 radiosensitivity 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 80–90% of the variation was due to deterministic effects related to the

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Presented at the 46th Annual Meeting of the American Society for Therapeutic Radiology and Oncology (ASTRO), Atlanta, GA, October 3–7, 2004 and at the 23rd Annual Meeting of the European Society for Therapeutic Radiology and Oncology (ESTRO), Amsterdam, The Netherlands, October 24–28, 2004.

This research was supported by Department of the Army grant DAMD 17-02-1-0503, the ESTRO GENEPI Project, and by the Danish Cancer Society.

Received Feb 11, 2005, and in revised form June 12, 2005. Accepted for publication Sept 6, 2005.

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 to have received significant attention was the mutated in ataxia telangiectasia (AT) gene, *ATM*, as it was reported more than 30 years ago that patients suffering from the disease ataxia telangiectasia exhibit unusually severe and devastating responses to ionizing radiotherapy (7, 8). The ATM protein functions primarily as a protein kinase involved in cellular stress responses, cell cycle checkpoint control, and deoxyribonucleic acid (DNA) repair (9). Evidence in support for the role of *ATM* genetic variants conferring radiosensitivity to breast cancer patients comes from a study (10) in which 46 breast cancer patients were screened for *ATM* sequence variations. It was reported that 100% (3/3) of the patients that developed a Grade 3/4 subcutaneous reaction, manifested as either fibrosis or soft tissue necrosis, had *ATM* missense mutations. A second study reported a significant association specifically between homozygote carriers of the G→A transition at *ATM* nucleotide 5557 and adverse radiotherapy responses (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 enables the rapid, sensitive, and accurate identification of genetic variants in an automated fashion. Of greatest importance is the evidence that DHPLC possesses 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 hypofractionated radiotherapy protocol. Because of a 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 have been 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 subcutaneous tissue toxicities, this Danish patient cohort represents a unique population because of the relatively large biologic doses received and the availability of skin biopsies. Furthermore, all patients in the study cohort were scored for subcutaneous fibrosis in three independent treatment fields. Differences in the dose distribution between these fields, as well as the diversity in fraction size used to treat the patients, resulted in substantial intra- as well as interpatient variation in biologically equivalent dose of 2 Gy per fraction, thereby permitting a dose–response analysis of these data. The high incidence of patients with late effects provides an ideal population to identify genetic factors associated with radiosensitivity because the doses used reached a level at which radiosensitive patients were likely to manifest a late radiation response. The relatively high biologic doses given to many patients in this cohort make this a relevant population to study in regard to treatment of tumors that require high doses to achieve control and therefore routinely result in normal tissue radiation doses in the 60–70 Gy range. In addition, the study cohort may be of particular interest considering the ongoing discussion about the ideal treatment technique (21) and fractionation regimen in postoperative radiotherapy for breast cancer (22, 23).

METHODS AND MATERIALS

Treatment characteristics, dose, and scoring of normal tissue reactions

Breast cancer patients were treated with postmastectomy radiotherapy in the Department of Oncology, Aarhus, Denmark from 1978–1982 using two fractionation protocols as previously described (19, 24). The 41 patients screened in this study represent a portion of the cohort of 319 breast cancer patients given postmastectomy radiotherapy during this period (25) and constitute the subjects for whom cultured fibroblasts were available (20). All patients were uniformly treated with a three-field technique comprising an anterior photon field, bolus area of the photon field, and an anterior electron field (Fig. 1). Thirty-four patients received 12 fractions to a minimum target dose of 36.6 Gy specified at the level of the mid-axilla or to an irradiated dose of 51.4 Gy irrespective of anteroposterior diameter. The other 7 patients were given a minimum target dose of 40.9 Gy in 22 fractions also specified at the mid-axilla. Every patient was evaluated for subcutaneous fibrosis in each individual treatment field at a single follow-up 2.2 to 5.4 years (median, 4.0 years) after completion of radiotherapy. Fibro-

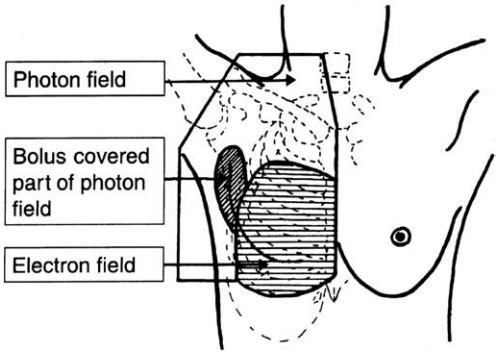

Fig. 1. Treatment field arrangement for postmastectomy radiotherapy in Aarhus 1978–1982. All patients screened in this study were treated with this technique.

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