Abnormal Expression of the ATM and TP53 Genes in Sporadic Breast Carcinomas

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ABSTRACT

The ataxia telangiectasia gene (ATM) has been implicated as a risk factor in the development of sporadic breast carcinomas. ATM protein expression was analyzed by immunohistochemistry in 17 breast carcinomas with two monoclonal antibodies whose immunohistochemical use was first validated by comparing the immunoreactivity observed in spleen samples from ataxia telangiectasia and trauma patients. In normal breast ducts, ATM showed nuclear expression in the epithelial but not in the myoepithelial cells. In contrast, this nuclear expression was absent or low in the epithelial cancer cells in 10 of 17 (59%) of the tumors studied. Allelic imbalance in the ATM gene was found in three of seven tumors examined. Two of these showed reduced ATM protein expression, but this did not correlate with the presence of ATM mutations in the tumor DNA detected by restriction endonuclease fingerprinting screening. These results suggest that the reduced ATM protein expression could be attributable, in certain tumors, to deletions or rearrangements within or close to the ATM gene. Positive p53 immunostaining was found in 10 tumors, with TP53 mutations detected in 8. Three tumors had both low ATM expression and mutated TP53. Our results indicate that in the majority (15 of 17) of the sporadic breast carcinomas examined, not only is the functionality of the ATM-p53-mediated DNA damage response compromised, but also other signaling pathways activated by these two multifunctional proteins are likely to be impaired, which could be a contributing factor to tumor development and progression.

INTRODUCTION

Several genes are known to predispose women to breast cancer, which is a common disease with a complex etiology. Although mutations in BRCA1 and BRCA2 are recognized as risk factors for inherited breast cancer, somatic mutations in these genes are rare in sporadic breast cancers (1, 2). In principle, a greater proportion of breast cancer cases within the population could be attributed to genes that are more frequently mutated but which may have a relatively low penetrance with respect to breast cancer. The AT1 gene (ATM) is one candidate for such a susceptibility gene.

AT is characterized by cerebellar ataxia, skin, and ocular telangiectasias, immunodeficiency, extreme cellular sensitivity to ionizing radiation, and predisposition to cancer. Epidemiological studies on AT families have shown that AT heterozygotes also have an increased risk of developing cancer, in particular breast cancer, for which female AT carriers have a 4-fold increased risk compared with the general population (3–5). It has been estimated that because ~1% of the general population are AT heterozygotes, alterations in the ATM gene could account for up to 8% of all breast cancer cases. A role for the ATM gene in sporadic breast cancer is supported by many studies that have shown a LOH in the region of the ATM gene located on chromosome 11q23.1. This has been found in ~40% of tumors studied (6–10). A causative association with the ATM gene has, however, been shown in only a few familial cases of breast cancer (11–13), and to date, few ATM mutations have been reported in sporadic or early onset breast cancer (for a recent review, see Ref. 14).

Normal breast tissue shows a distinct pattern of ATM expression, the protein being found in the ductal epithelial cells but not in the surrounding myoepithelial cells (15). In contrast, in cases of sclerosing adenosis, a benign breast lesion, ATM is expressed in both the epithelial and myoepithelial cells. This up-regulation of ATM expression was associated with proliferation of the myoepithelial cells (15). Recently, Kairouz et al. (16) have reported a reduction in the level of ATM protein in sporadic breast tumors. ATM mRNA levels have also been found to be lower in invasive breast carcinomas than in normal breast tissue or benign lesions. This reduction was observed in breast tumors with or without LOH in the region of the ATM gene, suggesting that genetic events other than gene deletions could result in reduced ATM gene expression (17).

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3 The abbreviations used are: AT, ataxia telangiectasia; LOH, loss of heterozygosity; TTGE, temporal temperature gradient gel electrophoresis; IDC, invasive ductal carcinoma; REF, restriction endonuclease fingerprinting.
Another gene in which mutations have been shown to predispose to breast cancer, especially associated with the Li-Fraumeni syndrome, is the \textit{TP53} gene. Using immunohistochemical approaches, elevated levels of the p53 protein have been found in a high percentage (20–55\%) of breast tumors. However, the prevalence of \textit{TP53} mutations in such sporadic breast tumors is lower (15–46\%), suggesting that in some tumors, it is wild-type p53 and not mutant protein that is being detected (18, 19). This apparent increase in protein expression may be a response to a variety of DNA stresses, including DNA damage, oxidative stress, or hypoxia.

The interaction between the ATM and p53 proteins is essential in the cellular response to DNA damage and in particular, double-strand breaks that can arise endogenously during cellular processes, such as V(DJ) recombination or meiosis, or after exposure to DNA-damaging agents, such as ionizing radiation. The signaling cascades that are activated in response to such damage remain to be fully elucidated, but both proteins play critical roles. In response to ionizing radiation, the kinase activity of ATM is enhanced, leading to phosphorylation of p53 on serine 15. The activated ATM kinase also phosphorylates and activates the checkpoint kinase Chk2, which in turn, phosphorylates p53 on serine 20 (20). These interactions result in the stabilization of p53 and its activation as a transcription factor of genes such as \textit{WAF1/Cip1}, \textit{MDM2}, \textit{GADD45}, \textit{BAX}, and \textit{IGF-BP3}. Their transcriptional activation is associated with cell cycle arrest, DNA repair, or apoptosis (21). ATM can also regulate the cell cycle by p53-independent pathways, involving c-Abl, replication protein A, and Chk2 (21, 22). Both of these multifunctional proteins are also involved in various other signaling pathways (23, 24). Thus, abnormalities in either ATM or p53 expression could have dramatic consequences for both the control of normal physiological processes and the cellular response to DNA damage. The aim of the present study was to investigate whether there was a correlation between the protein expression profiles of ATM and p53 and the mutational status of the \textit{TP53} gene in breast carcinomas using immunohistochemical techniques and DNA analysis.

\section*{MATERIALS AND METHODS}

\subsection*{Patient Samples.} This study was approved by the local ethical committees. Breast tissue was obtained from 17 unselected female patients undergoing surgery for breast cancer at the Centre Régional Léon Bérard (Lyon, France). Sixteen samples were IDCs according to WHO criteria. Two of these tumors were associated with an \textit{in situ} carcinoma. One sample was exclusively an \textit{in situ} ductal carcinoma. The grade of the invasive carcinomas was evaluated according to Scarff-Bloom-Richardson grading (25). None of the patients was treated with radiotherapy, but three patients had received treatment before surgery: one with chemotherapy, one with hormone therapy, and one with both (see Table 1). Six non-cancer (normal) breast samples from reduction mammoplasties were used as controls. All samples were snap-frozen in liquid nitrogen and stored at −70°C until further processing. Two formalin-fixed paraffin-embedded spleen samples from clinically diagnosed AT patients and two age-matched spleen samples from trauma patients were respectively used as negative and positive controls for the ATM staining.

\subsection*{Immunohistochemical Analysis for ATM and p53 Expression.} Frozen tissues were directly fixed in 4\% formalin for 12–24 h and then paraffin-embedded. For each sample, morphological assessment was carried out on a 4-μm tissue

\begin{table}
\centering
\caption{ATM immunostaining and \textit{TP53} status in breast carcinomas}
\begin{tabular}{|c|c|c|c|c|c|c|c|c|c|}
\hline
Sample no. & Histology & Grade & ATM expression & Immunostaining & Mutations$^a$ &
\hline
TS1$^c$ & IDC & III & Low & Intensity & % positive cells & CM1 & DO-7 & Codon & Exon & Base & AA change \\
TS2 & IDC & III & High & 10 & + & + & 174 del 18 & 5 & AGG$\rightarrow$GAG & Arg$\rightarrow$Glu \\
TS3 & IDC & III & Low & 50 & + & + & None & & & & \\
TS4 & IDC & I & High & $>80$ & + & - & None & & & & \\
TS5 & IDC & II & Moderate to high & $>80$ & - & - & None & & & & \\
TS6 & IDC$^+$ & III & Low to moderate & 80 & + & + & 248 7 & CGG$\rightarrow$TGG & Arg$\rightarrow$Trp \\
TS7 & IDC & II & Low & $>80$ & - & - & None & & & & \\
TS8 & IDC & II & Low & 20–50 & + & + & 248 7 & CGG$\rightarrow$TGG & Arg$\rightarrow$Trp \\
TS9 & IDC & II & Moderate & 50 & + & + & 273 8 & CGT$\rightarrow$CTT & Arg$\rightarrow$Leu \\
TS10$^b$ & IDC & III & Moderate to high & $>80$ & + & + & 248 7 & CGG$\rightarrow$TGG & Arg$\rightarrow$Trp \\
TS11 & IDC$^+$ & III & Low to moderate & 20–50 & + & + & 248 7 & CGG$\rightarrow$CAG & Arg$\rightarrow$Gln \\
TS12 & IDC & II & 0 & 0 & - & - & None & & & & \\
TS13 & DCIS$^*$ & Low & 20 & - & - & None & & & & \\
TS14 & IDC & II & 0 & 0 & - & - & None & & & & \\
TS15$^{a,b}$ & IDC & II & Low & 10–20 & + & + & 279 8 & GGG$\rightarrow$GAG & Gly$\rightarrow$Glu \\
TS16 & IDC & III & Low & 50–80 & + & + & 248 7 & CGG$\rightarrow$CAG & Arg$\rightarrow$Gln \\
TS17 & IDC & II & Low & 20 & - & - & None & & & & \\
\hline
\end{tabular}
\end{table}

\begin{itemize}
\item $a$ Patients received hormone therapy before surgery.
\item $b$ Patients received chemotherapy before surgery.
\item $c$ Associated with \textit{in situ} carcinoma.
\item $d$ Exons 4–9 screened.
\item $e$ DCIS, ductal carcinoma \textit{in situ}.
\end{itemize}
section stained with H&E, and the following sections were used for immunostaining. All tumor samples contained >80% tumor cells. The heat-induced epitope retrieval was optimized for both the spleen and breast tissues such that a distinct signal could be detected in the lymphocytes, which served as an internal positive control. After deparaffinizing, the endogenous peroxidases were inactivated by incubation for 30 min in 0.3% H2O2/methanol, and the slides were rehydrated. For ATM analysis, each section was then treated with antigen unmasking solution (Vector Laboratories Inc., Biosys S.A., Compiègne, France) either for three times for 5 min each in a microwave oven for the breast tissues or by pressure cooking for 10 min for the spleen tissues. The slides were allowed to cool in this solution and subsequently washed in PBS (three times for 5 min each). Antigen demasking was not necessary for p53 analysis. After blocking the nonspecific protein binding with PBS-5% skimmed milk-0.1% BSA for 45 min, the slides were incubated overnight at 4°C with the primary antibody. For ATM detection, two different monoclonal antibodies were used: ATM1 (ATM132) or ATM2 (ATML2; 1/20 dilutions) raised against amino acids 819–844 and 2581–2599, respectively (Ref. 26; generously provided by Dr. Yosef Shiloh, Tel Aviv University, Ramat Aviv, Israel). For p53 detection, the polyclonal CM1 antibody (1/500 dilution; Novocastra Laboratories Ltd, Newcastle, United Kingdom) and the monoclonal DO-7 antibody (1/50 dilution; Dako S.A., Trappes, France), which both recognize wild-type and mutant forms of the human p53 protein, were used. All of the antibodies were diluted in PBS-0.1% BSA. After PBS washings (three times for 5 min each), slides were incubated for 45 min with the secondary antibody: an antimouse (ATM1, ATM2, and DO-7) or an antirabbit (CM1) biotinylated serum (Vectastain ABC Kit, Vector Laboratories Inc.; 1/200 dilution; Dako S.A., Trappes, France), which both recognize wild-type and mutant forms of the human p53 protein, were used. All of the antibodies were diluted in PBS-0.1% BSA. After PBS washings (three times for 5 min each), slides were incubated for 45 min with the secondary antibody: an antimouse (ATM1, ATM2, and DO-7) or an antirabbit (CM1) biotinylated serum (Vectastain ABC Kit, Vector Laboratories Inc.; 1/200 dilution in PBS-0.1% BSA). The sites of peroxidase binding were revealed by diaminobenzidine staining (Vector Laboratories Inc.) after streptavidin signal amplification (Vectastain ABC Kit, Vector Laboratories Inc.). Mayer’s hematoxylin was used for counterstaining before dehydration and mounting of slides. In sections used as negative controls, the primary antibodies were omitted. The cellular localization, intensity, and the percentage of cells with positive ATM staining were assessed on the whole section by two investigators. In the case of p53, tumors were considered as immunohistochemically positive when >10% of the tumor cells showed nuclear p53 staining.

DNA Extraction and Microsatellite Analysis. For seven tumors, DNA could be extracted from both the tumor and the surrounding noninvolved normal tissue by microdissection. For each tissue sample, four 5-μm sections were deparaffinized, and the appropriate areas of tissue were incubated at 55°C in 50 μl of TE buffer [10 mm Tris, 1 mm EDTA (pH 9.0)] containing 0.1 μg/μl proteinase K and 0.25% NP40. Proteinase K, at the same concentration, was added every 12 h until tissue digestion was complete and then inactivated by heating at 95°C for 10 min. Samples were centrifuged for 2 min at 10,000 rpm, and the supernatant was stored at 4°C.

Allelic imbalance was determined at the ATM locus using two intragenic microsatellite markers, D11S2179 and NS22, and three closely flanking markers, D11S1819, D11S1294, and D11S1818. Primer sequences and PCR conditions for the NS22 marker (ATM7F and NS22R primers) were those of Udar et al. (27) and for all of the other microsatellite markers as described in the Genome Database.4 One 5’-nucleotide of each primer pair was labeled with a different fluorescent dye. For each sample, PCR products obtained for the five different microsatellite markers were mixed and denatured at 95°C for 2 min. Electrophoresis was carried out on a 4.8% denaturing polyacrylamide gel in the presence of 0.5 μl of a TAMRA-labeled size standard (Genescan-500 TAMRA, Applied Biosystems, Courtaboeuf, France) in a model 377 DNA Sequencer (Applied Biosystems) for 4 h at 1000 V. All samples were processed at least twice. Collected data were analyzed using the Genescan Analysis 2.1 software. A difference in the allele ratios in tumor DNA compared with normal tissue DNA ≥30% was scored as allelic imbalance, which could thus correspond to a gain or a loss of one allele.

TP53 Mutation Detection. The TP53 mutational status of all samples was analyzed by TTGE, which can be used to screen DNA fragments for small sequence changes or point mutations. DNA was extracted from 25 mg of frozen tissue using the DNeasy Tissue Kit (Qiagen S.A., Courtaboeuf, France). To avoid any possible cross-contamination, each frozen tissue sample was cut and weighed separately using disposable, sterile scalpels and dishes. Gloves were changed between handling each tissue sample. Exons 4–9 of the TP53 gene were amplified using primers with a GC-rich sequence as described by Hamelin et al. (Ref. 28; exons 5–8) and Guldberg et al. (Ref. 29; exons 4 and 9). Heteroduplex formation was induced by a 10-min incubation at 98°C and then 30 min at 55°C or 62°C (depending on the exon being screened). Samples were electrophoresed in a 7.5 or 9% polyacrylamide gel containing 7 or 8 m urea, respectively, in 1.25% TAE [50 mM Tris acetate, 25 mM sodium acetate, 1.25 mM EDTA (pH 7.4)]. Migration was carried out at 130 V with a temperature range between 53°C and 70°C and a rate of change of temperature of 2°C or 3°C/h according to the exon being analyzed.5 Samples with aberrantly migrating bands were reamplified and a second TTGE was performed. Mutant alleles were cut from this second gel and reamplified using the appropriate primers. For direct sequencing of this PCR product, asymmetric PCR amplifications were performed as previously described (30). Reactions were run on polyacrylamide-urea gels, dried, and exposed to Kodak BioMax MR-1 autoradiography films. The mutation spectra found were compared with those reported in the IARC TP53 mutation database.6

RESULTS

Immunohistochemical Characterization of ATM Expression. The two ATM monoclonal antibodies used in this study each recognize a Mr 370,000 protein on Western blots of protein extracts prepared from normal cells and AT cells ectopi-

4 Internet address: http://www.gdb.org/gdb/.
cally expressing ATM, but this protein is absent in AT cells (26). The comparison of the staining pattern in tissue sections from two AT patients and two control subjects validated the use of these two antibodies for immunohistochemical detection of ATM in tissue sections: both antibodies gave identical results. The lymphocytes in the spleens of the control subjects showed positive ATM immunostaining (Fig. 1, a and c) but not the lymphocytes in either of the tissue sections from the AT patients (Fig. 1, b and d). Although the exact nature of the ATM mutations in these AT patients is not known, they showed all of the characteristic clinical symptoms of the disease. The vast majority of such patients have null mutations in the ATM gene, which are predicted to give rise to a truncated protein that is unstable and not detected by immunological techniques (31).

In the six normal breast tissue samples examined, the ATM protein was detected in the nucleus of the epithelial cells but was absent in the ductal myoepithelial cells (Fig. 2, a--c). This profile is identical to that previously reported for normal breast tissue (15, 16). In contrast, in 56% of the IDCs examined (9 of 16), the nuclear ATM staining in the epithelial cancer cells was absent or at a low intensity compared with that seen in the normal epithelial breast cells (Fig. 2, e--g and Table 1). Seven of 17 tumors expressed ATM protein at moderate to high levels. In some cases, the ATM expression was similar to that seen in normal epithelial cells: these latter cases are graded as “high” in Table 1. Two of 17 breast tumors exhibited no detectable ATM expression, although ATM expression could be detected in the lymphocytes within the same tissue section. The only tissue sample, which was exclusively an in situ carcinoma, showed a low intensity of nuclear ATM staining in ~20% of the tumor epithelial cells. In the two cases in which in situ carcinoma was present together with the invasive component, ATM expression was identical in both lesions.

The percentage of epithelial cells expressing ATM protein was variable between tumors. However, even in those sections where a high percentage of the cells expressed ATM, there was very little inter-cell variation in the intensity of staining (Fig. 3 and Table 1). In six of 17 tumors, general cytoplasmic ATM staining was observed. This was also present in the negative control for each of these six samples where no primary antibody was used; thus, this cytoplasmic ATM staining must be considered as nonspecific (data not presented).

Allelic Imbalance in the Region of the ATM Gene.

Seven of 17 primary breast carcinomas from which noninvolved and tumor tissue could be isolated by microdissection were selected to study allelic imbalance in the region of the ATM gene. The NS22 marker, which was previously shown to be a highly informative marker in the general population (27), was uninformative in six of seven cases examined in this study. Three breast tumors (TS5, TS8, and TS13) demonstrated allelic imbalance at the D11S2179 locus located within the ATM gene, and this was associated with allelic imbalance of a flanking marker, either centromeric (D11S1819) or telomeric (D11S1294 and D11S1818). This allelic imbalance correlated in TS8 and TS13 with a low level of ATM protein expression in ~20% of the tumor cells and thus could be considered as an allelic loss. In contrast, in TS5, a moderate to high ATM staining in >80% of the tumor cells was observed, suggesting a gene amplification or rearrangement. The entire ATM coding sequence of TS5, TS8, and TS13 was analyzed for the presence of mutations using the REF technique (32, 33), and no ATM mutations were detected (data not presented). In the other four samples, there was

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7 S. Becker-Catania, personal communication.
no clear correlation between ATM expression and allelic imbalance in the region of the ATM gene.

**Immunohistochemical Characterization of p53 Expression.** Positive nuclear p53 immunostaining was detected in 9 of 16 (56%) of the IDCs examined using the monoclonal antibody DO-7. One additional sample showed positive nuclear staining in ~20% of the tumor cells when the polyclonal antibody CM1 was used (Table 1). Cytoplasmic staining was not observed in this series of samples. Among the 10 tumors exhibiting no detectable or a low level of ATM staining, six showed a negative nuclear p53 staining and four had positive nuclear p53 staining (Fig. 2h) when compared with the normal tissue (Fig. 2d).

TP53 Gene Mutations. Exons 4–9 of the TP53 gene were analyzed using TTGE. Eight of 10 tumors with positive p53 immunostaining contained a TP53 mutation (Table 1). Seven of these mutations were missense mutations, and the majority were located in exons 7 and 8. Three tumors contained the same point mutation, CGG→TGG (Arg→Trp) at codon 248 in exon 7. A different point mutation at the same codon was also observed in two other tumors (CGG→CAG, Arg→Gln). These mutations at codon 248 were reconfirmed using a second DNA sample extracted independently from each of the five tumor samples. One tumor contained an in-frame 18-nucleotide deletion at codon 174 in exon 5. No mutations were detected in the seven tumors showing negative p53 immunoreactivity with the CM1 and/or DO-7 antibodies.

**DISCUSSION**

Using the immunohistochemical technique, we have demonstrated a specific ATM expression profile in normal breast tissue with nuclear expression in the duct epithelial cells, which is absent in the myoepithelial cells. Using two ATM antibodies, which recognize different epitopes on the ATM protein, we found a significant reduction in the intensity of the nuclear ATM staining in the epithelial cancer cells in 59% of the breast tumors examined. These results are in agreement with the recent report by Kairouz et al. (16), who showed a reduction or absence of ATM protein immunoreactivity in 14 of 42 invasive breast ductal carcinomas examined. Interestingly, they also noted that the levels of ATM were greatly reduced or absent in most metastatic breast carcinomas in lymph nodes (10 of 14), compared with the levels observed in nonmetastatic invasive breast carcinomas. A consistent trend was found toward weaker ATM immunostaining for more invasive stages between patients and among all patients with progressive stages of breast cancer. This observation may in part explain the higher percentage of IDCs with reduced ATM expression seen in our study because 7 of 16 examined were grade III. This reduced ATM expression in breast carcinomas is also consistent with the decrease of ATM mRNA transcript levels in breast carcinomas compared with benign lesions and normal breast tissue reported by Waha et al. (17).

Although the different studies have found a similar cellular profile of ATM expression in normal breast tissue and a reduction in its expression in some breast tumors, significant differences have been noted in the extent of cytoplasmic staining found both in normal and breast cancer cells. In the present study, cytoplasmic ATM immunostaining was noted in 6 of 17 tumors but not, in those cases where it could be examined, in the surrounding noninvolved epithelial cells or in any of the normal breast tissues examined. In all of these six tumors, this cytoplasmic staining pattern was still observed in the tumor when the primary antibody was omitted, but the lymphocytes, which served as internal positive controls for ATM expression, showed no staining under these conditions. These observations strongly suggest that the cytoplasmic staining seen in some tumor tissues was nonspecific. However, in the study by Kairouz et al. (16), strong immunoreactivity was seen using antibody ATM-4BA in the cytoplasm of cells within the inner epithelial layer of normal ducts in the non-neoplastic normal-appearing breast tissue in 29 of 36 samples, whereas 7 of 36 showed nuclear staining. A similar staining pattern was reported in the tumor tissues, with some variation in both the intensity and extent of immunostaining (16). The reasons for these differences in the cytoplasmic staining pattern are unclear. Both the antibodies used in
immunostaining using the ATM1 or the ATM2 antibodies is absent (a3 panels, b-adaptin (35). To the vesicles in the cytoplasm (34), where it interacts with fibroblasts, ATM is expressed in the nucleus but also localizes to the cytoplasm. To date, no such modification of the ATM protein in the translocation of the protein from the nucleus to the cytoplasm has been reported. In both lymphoblastoid cells and primary fibroblasts, ATM is expressed in the nucleus but also localizes to the vesicles in the cytoplasm (34), where it interacts with β-adaptin (35). β-Adaptin is one of the components of the AP-2 adaptor complex, which is involved in clathrin-mediated endocytosis of receptors. This interaction between ATM and β-adaptin may play a role in the regulating mechanisms of vesicles and/or protein transport. It has been recently shown, using the ATM4-BA antibody, that a portion of ATM co-localizes with the peroxisomal matrix protein catalase (36).

There are many potential molecular causes for a reduction in ATM expression. Several regions on chromosome 11 have been identified, which are frequently lost in breast tumors (37–42). In this study, allelic imbalance on chromosome 11 was found in five of seven tumors and occurred within the ATM gene in three cases. Interestingly, low levels of ATM expression were found in two of these three cases; thus, the reduction could be explained by loss of an ATM allele. However, because no mutations were detected in these samples, it would seem that either both ATM alleles were wild-type or the mutated allele was lost in the tumor. Relatively few studies have characterized the mutational status of the ATM gene in breast tumors. Chen et al. (12) examined the tumor tissue from four AT heterozygotes identified in their study population of 188 patients with a family history of breast cancer. In three of these tumors, both the mutated and wild-type alleles were retained, and in one, the mutated allele was lost, suggesting that the ATM gene was not involved in the pathogenesis of these familial breast cancers. In contrast, Bay et al. (13) reported the loss of the wild-type ATM allele in the breast tumor tissue of an AT heterozygote identified in a family with a high cancer incidence. This observation would support the hypothesis that haplo insufficiency at ATM may promote tumorigenesis and a more classic role for ATM as a tumor-suppressor gene. A role for the ATM gene in breast cancer development is also supported by the recent results of Izatt et al. (43) who found that in five breast tumors from early-onset breast cancer patients with germ line rare ATM missense sequence variants, the wild-type allele was lost.

The ATM expression profile in breast tumors resembles that previously reported for BRCA1. Several studies have shown that BRCA1 gene expression at the mRNA level (44–46) and at the protein level (47–48) is significantly reduced in some sporadic breast tumors. Interestingly, low levels of BRCA1 expression were also detected in sporadic breast tumors irrespective of whether LOH in the BRCA1 gene was found (49). Thus, it appears that the expression of BRCA1, and by analogy ATM, can be regulated by different mechanisms, including allelic loss and modulation of the protein expression at the transcriptional and translational levels in sporadic breast carcinomas. In the case of BRCA1, abnormal methylation of the BRCA1 gene promoter region and preferential allelic expression have been reported in sporadic breast tumor samples (46, 50, 51). It remains to be determined whether altered methylation patterns of the ATM promoter can explain the reduced ATM expression seen in breast tumors. Luo et al. (52) have shown that in lymphocytes expressing ATM, the promoter region is completely demethylated. However, they were unable to correlate the methylation status and the variable ATM protein expression observed in the T-PLL tumor samples in which no ATM mutations were found.

The second expression profile that was examined in these breast tumors was that of the TP53 gene. The frequency of tumors with p53 overexpression (59% with the CM1 antibody) and that with TP53 mutations (47%) are at the higher end of the ranges reported where large cohorts of randomly selected breast cancer cases have been examined (18). Although TP53 mutations have been detected at the earliest clinical stages of neoplastic transformation, the fraction of tumors with an altered TP53 gene is typically higher in late-stage tumors. In breast tumor tissue, a significant correlation between the presence of
ATM and TP53 in Sporadic Breast Carcinomas

Table 2  Classification of 17 tumors by factors affecting ATM- and p53-dependent signaling pathways

<table>
<thead>
<tr>
<th>TP53 mutated</th>
<th>ATM expression absent or low</th>
<th>Tumors with compromised ATM or p53 signaling pathways</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>10</td>
<td>15/17 tumors*</td>
</tr>
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</table>

* Three tumors had both a TP53 mutated and a low ATM expression.

TP53 mutations and a high histological grade (P < 0.001) has been reported (53). This may explain the high prevalence of TP53 mutations found in this study because 7 of 16 of the samples analyzed were grade III IDCs, five of which had a mutated TP53 gene.

The vast majority of the TP53 mutations detected in this study are among those frequently found and are in accordance with the published TP53 mutation spectra in breast cancer. They were all clustered in the core domain of p53, which contains the sequence-specific DNA-binding activity of the p53 protein (residues 102–292). This binding activity is critical for p53’s role as a transcriptional activator. Arginine residues in this core domain play a particularly important role in protein-DNA contacts (Arg248 and Arg273) or in stabilizing the structure of the DNA-binding surface of p53 (Arg175, Arg249, and Arg 282; Ref. 54). The only TP53 mutation found in this series of samples, which did not involve an arginine residue, GGG→GAG at codon 279 in exon 8, which will result in a glycine→glutamic acid amino acid change, has not been reported in the IARC TP53 mutation database in breast tumors.

By examining both ATM and p53 profiles in the same tumor sections, this study has revealed for the first time an interesting relationship between the expression of these two multifunctional proteins in normal compared with tumor tissue (Table 2). In the 17 breast tumors analyzed in this study, 10 cases exhibited no detectable or low ATM protein expression and 8 tumors contained a mutated TP53, which was associated in three samples with low ATM protein expression. Thus, in 15 of 17 of the sporadic breast carcinomas examined, the functionality of the intricate web of signaling pathways activated in response to DNA damage is likely to be compromised. In those tumors expressing a low level of ATM protein, it might be expected that the activation of p53 through its phosphorylation by ATM’s kinase activity in response to certain forms of DNA damage or stresses will be suboptimal. Indeed, one of the hallmark features of AT homozygote cells is reduced and delayed p53 induction after exposure to ionizing radiation (55, 56). In lymphoblastoid cell lines from AT heterozygotes, suboptimal induction of p53 has also been found (13, 57).8 A low level of ATM expression may also influence the modification of other physiological targets that are phosphorylated by the ATM kinase. In response to DNA double-strand breaks, the activated ATM kinase phosphorylates and activates the checkpoint kinase Chk2 (20). These activated kinases phosphorylate p53 on serines 15 and 20, respectively, which results in its stabilization and activation of its transcriptional activity. Khosravi et al. (58) have demonstrated that in response to DNA strand breaks, ATM also phosphorylates Mdm2, which itself negatively regulates p53’s stability and activity and whose gene is activated by p53. Mdm2 binds to the amino-terminus of p53, represses its transcriptional activity, and targets it to proteasome-mediated degradation (59–61). Thus, ATM may promote p53 activity and stability by mediating simultaneous phosphorylation of both partners of the p53-Mdm2 autoregulatory feedback loop. A further target, clearly implicated not only in the etiology of breast cancer but also in transcription as a transcription factor and in DNA repair through its association with Rad51/BRCA2, is the BRCA1 protein (see Ref. 62 for a recent review). ATM is required for the phosphorylation of BRCA1 in response to ionizing radiation (63). Several other putative substrates have been identified, including p95/nibrin, which is altered in Nijmegen Breakage Syndrome patients, and its protein partner Mre11, suggesting that ATM may also regulate the function of the p95-Mre11-Rad50 repair complex in response to DNA damage (64). The presence of a mutated form of the p53 protein will also result in failure to activate the cellular responses normally triggered through p53 acting as a transcription factor. This possible inability of breast tumor cells to activate these various signaling pathways because of alterations in the levels of these two multifunctional proteins could be an important event in the development of sporadic breast tumors and their progression by increasing genomic instability and must now be examined in a larger study.

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ATM and TP53 in Sporadic Breast Carcinomas


Abnormal Expression of the \textit{ATM} and \textit{TP53} Genes in Sporadic Breast Carcinomas

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