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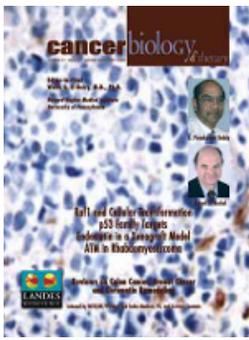
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Research Paper

Association of Ataxia Telangiectasia Mutated (ATM) Gene Mutation/Deletion with Rhabdomyosarcoma

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ABSTRACT

Rhabdomyosarcoma is a common malignancy in children. There are two major types of rhabdomyosarcomas, the embryonal and the alveolar, differing in cytogenetic and morphologic features. The alveolar type of rhabdomyosarcoma is frequently associated with chromosome translocation t(2; 13) and poor clinical prognosis. Pathogenesis of rhabdomyosarcoma remains obscure, and especially it occurs in the location where skeletal muscle is absent. We report here that there is a high frequency of association of rhabdomyosarcoma with ataxia telangiectasia mutated (ATM) gene mutation/deletion. Totally 17 cases of rhabdomyosarcoma specimens were studied by immunohistochemical or immunofluorescent staining with ATM antibody and revealed that 7 of the 17 cases were negative for ATM expression (41%). Further analyses of rhabdomyosarcoma cell lines with RT-PCR revealed that in Rh30 cells, an alveolar rhabdomyosarcoma cell line, there are three separate deletions/mutations of the ATM mRNA. Western blotting analysis of the Rh30 cellular extract with anti-ATM antibody showed that there is an aberrant form of ATM protein within the Rh30 cells that are smaller than normal control. These results suggest, for the first time, a link of ATM gene deletion/mutation with rhabdomyosarcoma, and since ATM kinase is a crucial regulatory protein in DNA damage repair signaling pathway, and ATM deletion/mutation may contribute to pathogenesis of rhabdomyosarcoma.

INTRODUCTION

Rhabdomyosarcoma is a malignant neoplasm of skeletal muscle, and is one of the most common soft tissue malignancies in the children and adolescent population. Histopathologically rhabdomyosarcoma can be divided into two major subtypes, embryonal rhabdomyosarcoma representing about 80% of the clinical cases, and alveolar rhabdomyosarcoma representing about 20% of the total cases.[1,2] Extensive cytogenetic studies showed that the embryonal rhabdomyosarcomas are generally associated with loss of heterozygosity (LOH) for multiple closely linked loci at chromosomal 11p15.5, suggesting inactivation of one or more tumor-suppressor gene(s) in this region.[2,3] But the gene involved in this region has yet to be identified. Many other associated mutation or amplification of oncogenes, tumor suppressor genes including *p53*, *K-ras*, *N-ras* and *N-myc*, etc. are frequently seen, but the roles of these gene mutations/amplifications in pathogenesis of the tumor remain largely unclear.[4,5] The alveolar subtype of rhabdomysarcoma, however, is consistently associated with two common chromosomal translocations. Most cases of alveolar rhabdomyosarcoma are associated with a chromosomal translocation t(2; 13) (q35; q14), and less commonly with t(1; 13)(p36; q14).[6-9] The t(2; 13) translocation results in fusion of a portion of the *Pax3* gene to a portion of the FKHR gene, leading to formation of fusion protein Pax3-FKHR. *Pax3* gene encodes a transcriptional factor important in early development program, while FKHR gene encodes another generic transcriptional factor that is widely expressed in mammalian tissues. The t(1; 13) translocation results in formation of Pax7-FKHR. Both Pax3-FKHR and Pax7-FKHR were shown to possess more potent transcriptional activity than its original forms Pax3 and Pax7.[10,11] How the chimeric fusion proteins contribute to pathogenesis of rhabdomyosarcoma remains unclear.

Ataxia telangietasia mutated gene (ATM) is a newly identified member of PI3 kinase family that is mutated in human autosomal recessive disease, ataxia telangiectasia (AT).[12,13] ATM gene is large, spanning about 150 kbs of genomic DNA, and is located on chromosome 11q22-23. In response to DNA damage agents such as ionizing irradiation and chemical agent cisplatin, ATM kinase is activated, leading to a cascade of kinase reactions regulating cell cycle, apoptosis, and DNA damage repair.[14,15] Many downstream target molecules of ATM kinase were so far identified. These target proteins include the c-Abl

tyrosine kinase, the p53 tumor suppressor, the Chk1 and Chk2 serine/threoine kinase, the p34 subunit of replication protein A (RPA), Chk1 serine/threoine kinase and NBS1 kinase which is mutated in human autosomal recessive disease Nijmegan breakage syndrome.[16-23] Clinical characteristics of AT patients include hypersensitivity to irradiation, thymic dysplasia, immune defects and higher tendency to develop malignant tumor, suggesting that ATM function is critical in DNA damage repair and tumor suppression. However, up to date, AT patients are not known to develop rhabdomyosarcoma.

In this study, we report an association of human rhabdomyosarcoma with deletion/mutation of ATM gene. This is the first report so far of ATM gene associated with solid malignancy except for the mantle cell lymphoma, a malignant tumor in lymphoid origin. The association of ATM gene mutation/deletion with rhabdomyosarcoma suggests a link of ATM gene with cancer risk, and ATM kinase may contribute to pathogenesis of rhabdomyosarcoma.

MATERIALS AND METHODS

Immunohistochemical and Immunofluorescent Staining. Human rhabdomyosarcoma tumor specimens were obtained from the Department of Pathology, the Children's Hospital San Diego. All human tissues were formalin fixed, paraffin embedded and were previously used for diagnostic purposes. Current study was approved by the Institutional Review Board at University of California, San Diego (IRB). The specimens were sectioned at 5 micron in thickness, and used for immunohistochemical staining with ATM antibody as described.[18] Briefly, the sections were de-paraffinized in xylene solution, dehydrated and rehydrated in 100%, 90% and 70% alcohol. The specimens were immersed in Antigen Retrieval solution (commercially available from Dako Corp. CA) at 90°C for 30 minutes to unmask the antigen followed by incubation with primary antibody against ATM protein at 40°C overnight. The primary anti-ATM antibody was kindly provided by Dr. Gately and as described previously.[18] The second antibody was either immunoperoxidase conjugated for immunochemical stains or Cy3 conjugated anti-rabbit IgG for immunofluorescent stains (Molecular Probes, OR). For immunofluorescent staining for the tumor cell lines, the tumor cells were cultured on the glass coverslips, fixed by 4% paraformaldehyde and stained with primary antibody against ATM and MyoD as described.[24]

Cell Culture, RNA Isolation, Reverse Transcription and PCR. Rhabdomyosarcoma cell lines, such as Rh3, Rh4, Rh 28, RH30 and RD cells were obtained from Dr. Karen Arden, Ludwig Institute of Cancer Research at UCSD. The tumor cell lines were cultured and maintained as described.[24] The total RNA was isolated from the cultured cells using Trizol RNA isolation kit (GBCOL-BRL, Besthesda, MD). The cDNA synthesis was performed using the cDNA synthesis kit (Superscript, GBCOL-BRL) using random hexamer and oligo-d(T) primers following the instruction from the manufacturer. The total cDNA was directly used as templates for PCR amplification for ATM gene using the primer pairs in Table 1 The PCR products were electrophoresed in 1% agarose gel and visualised using ethidium bromide.

Western Blotting of ATM Protein. The rhabdomyosarcoma cells are cultured and maintained as described.[24] The cellular proteins were prepared, separated on 4% polyacrylamide gel and transferred onto nitrocellulose membrane. The immobilized proteins were incubated with anti-ATM antibody.[18] The proteins of interest were detected by enhanced immunochemiluminescence kit from Amersham Life Sciences.[24]

Table 1 — OLIGONUCLEOTIDE PRIMERS FOR RT-PCR AMPLIFICATIONS

Primer	Oligonucleotide Sequence (5'–3')
1A (exon 36)	CTT CAG TFF ACC TTC ATA ATG C
1B (exon 43)	CCA TAC AAA CTA TCT GGC TCC
2A (exon 29)	CAG AGA TTG TGG TGG AGT TAT TG
2B (exon 36)	GCA TTA TGA AGG TCC ACT GAA G
3A (exon 42/43)	CTG GAA TAA GTT TAC AGG ATC TTC
3B (exon 51)	GAT GAT TTC ATG TAG TTT TCA ATT C
4A (exon 56)	AAG ATG TTG TTG TCC CTA CTA TG
4B (exon 65)	AAG GCT GAA TGAAAG GGT AAT TC
5A (exon 51)	GAT GGA GAA TGA AAG GGT AAT TC
5B (exon 57)	AGT CAC CAG ATT TCC ATA TTC TC
6A (exon 22)	CTA GGT CAA AGC AAT ATG GAC TC
6B (exon 30)	CAT GCG ATG GAA AAT GAG GTG
7A (exon 13)	TGC CTC CAA TTC TTC ACA G
7B (exon 22/23)	CCT CTC CTT TGT TAG ATG CC

RESULTS

Totally 17 patients specimen were retrieved from the archived tissue blocks. The clinical characteristics of these patients were listed as in Table 1. Some patient's clinical information was not available at the time of the study. These patients all carried clinical histopathological diagnoses of rhabdomyosarcoma (embryonal or alveolar type), and showed immunostaining positivity for myogenin, a specific skeletal muscle marker. Immunofluorescent or immunohistochemical staining with anti-ATM antibody was performed on the total of 17 specimens, and 7 of the 17 rhabdomyosarcomas were negative for ATM protein (41%) (see Table 1). Totally 10 of the 17 were positive for ATM kinase (59%). ATM protein was predominantly

Table 2 — RMS PATIENTS AND IMMUNOSTAINING PROFILES

| Case No.	Patients	Tumo location	Subtype	Signal intensity and location
1	15/M	paratesticular	embryonal	ATM (–)
2	19/M	prostate/bone marrow	alveolar	ATM (+)/weakly/cytoplasmic
3	4/M	salivary gland	embryonal	ATM (+)/cytoplasmic
4	3/M	pelvis	embryonal	ATM (+)/cytoplasmic
5	4/M	paraspinal	alveolar	ATM (+)/weakly/cytoplasmic
6	4/M	skull base	embryonal	ATM (+)/cytoplasmic
7	6/M	abdomen	alveolar	ATM (–)
8	unknown	unknown	alveolar	ATM (+)/cytoplasmic
9	unknown	unknown	embryonal	ATM (+)/cytoplasmic
10	unknown	unknown	embryonal	ATM (+)/cytoplasmic
11	unknown	unknown	embryonal	ATM (–)
12	unknown	unknown	unknown	ATM (–)
13	unknown	unknown	unknown	ATM (–)
14	unknown	unknown	unknown	ATM (–)
15	unknown	unknown	unknown	ATM (–)
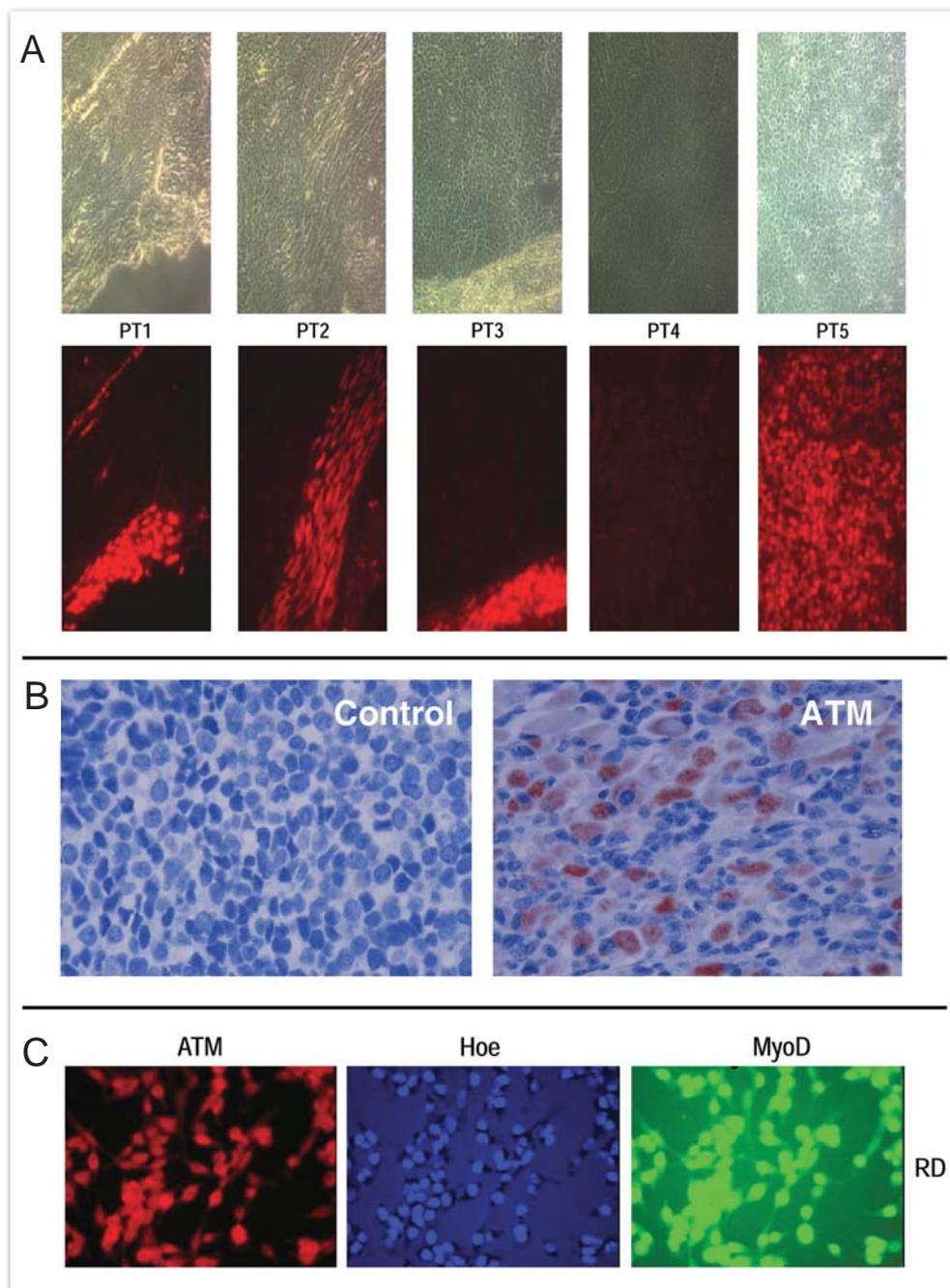
Figure 1. Immunostaining of rhabdomyosarcoma cases. (A) Immunofluorescent staining of five representative cases with anti-ATM antibody. PT: patient. The secondary antibody was conjugated to rhodamine. The positive signals were red fluorescent. The photograph was taken at 200X using Zeiss UV fluorescent microscope. (B) Immunoperoxidase staining of the rhabdomyosarcoma tumor with negative control. The positive staining was seen in cytoplasm. (C) Immunofluorescent staining of the cultured RD cells with antibodies against ATM and MyoD. The signals were seen in the nuclei. The photograph was taken at 400X.

located in cytoplasm in the tumor sections and interestingly, predominantly seen in the nuclei in the rhabdomyosarcoma cell line, as those seen with myoD staining (RD cells) (Fig. 1A and Fig. 1B vs. Fig. 1C). There is no difference in staining pattern in regard to the subtypes of the rhabdomyosarcomas, namely embryonal or alveolar subtypes.

It is surprising to note that 7 of the 17 (41%) rhabdomyosarcoma specimens stained negatively for the presence of ATM protein. Under the normal condition, ATM expression is ubiquitous. To further confirm the immunostaining results, two different approaches were taken. One was to see if the ATM mRNA is properly transcribed, and another is to directly see the ATM protein itself by Western blotting analysis from the tumors and compare with the normal form. Due to the limited resource of the fresh tumor samples, we turned to the cell culture system and used the well-established rhabdomyosarcoma cell lines. First, we designed eight pairs of oligonucleotide primers encompassing the almost entire coding sequence of ATM mRNA based on the published cDNA sequence (Genbank accession number U33841)25 (see Table 2). The primer designing strategy is shown in Figure 2A. ATM mRNA is approximately 9.4 kbs in length and contains multiple protein binding domains for its kinase activity and interaction with other cellular proteins (Fig. 2A). We used reverse transcription-PCR method to analyze three different rhabdomyosarcoma cell lines, RD cells (embryonal), Rh28, Rh30 (alveolar), and mouse normal myoblast C2C12 cells. The
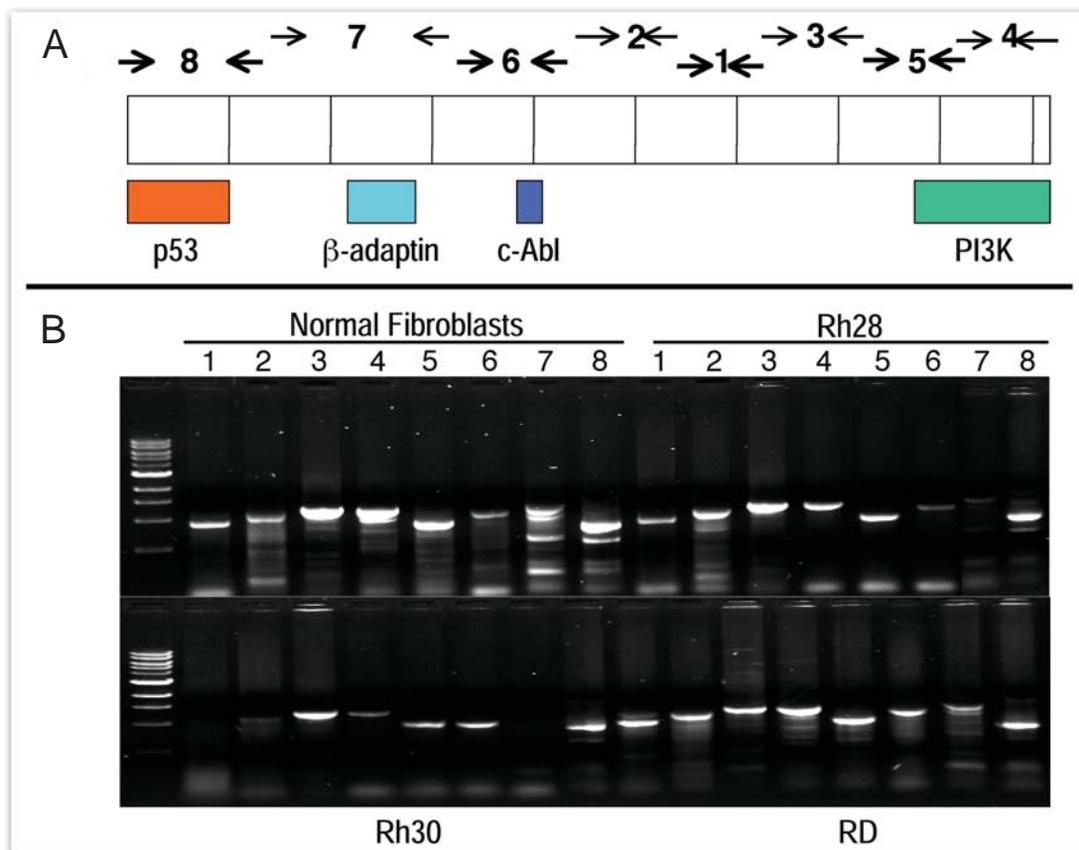
Figure 2. (A) Diagrammatic representation of ATM structure and RT-PCR strategy. The primer pairs were designed as shown. (B) RT-PCR amplification of ATM mRNA in the different cells.

human foreskin fibroblasts were used as the control cells. The mRNA was isolated from these cells and used for RT-PCR assays. As shown in Figure 2B, in normal fibroblasts, RD cells, Rh28 cells, the amplified DNA fragments were identical in regard to the lengths in comparing with the control fibroblasts, whereas in Rh30 cells, two fragments in #1 and #7 lanes (0.94 kbs in Lane #1, and 1.3 kbs in Lane #7) were absent, and in Lane #6, the fragment amplified was approximately 200 bps smaller than that seen in the fibroblasts and other rhabdomyosarcoma cells (Fig. 2B). The combined length of missing DNA sequence is estimated 2.4 kb (fragment #1, plus fragment #7, and additional 200 bps in Lane #6).

The next question is whether ATM protein is normally translated from ATM mRNA and expressed in the rhabdomyosarcoma cells. We analyzed the ATM protein in different cell lines. In human Hela cells and human normal foreskin fibroblasts, the ATM was estimated to be 360 kD, consistent with the results previously described.[12,13] In three rhabdomyosarcoma cell lines (RD, Rh28 and RH30), there was one aberrant form of ATM protein (~260 kD) that was significantly smaller than that seen in the normal fibroblasts and the Hela cells (Fig. 3). The levels of ATM expression in these three rhabdomyosarcoma cell lines are not identical, and Rh30 cells seemed to express less ATM than the other two Rhabdomyosarcoma cells. The murine ATM proteins from C2C12 and 10T 1/2 cells were significantly different in size and there appeared to be two species of ATM proteins in murine cells.

DISCUSSION

ATM gene is a member of PI3 kinase family. ATM kinase is critical in DNA damage signaling pathway. In response to DNA damage induced by ionizing radiation or chemical agents such as cisplatin, ATM kinase is activated and phosphorylates a number of downstream regulatory proteins important for cell cycle arrest, growth control,
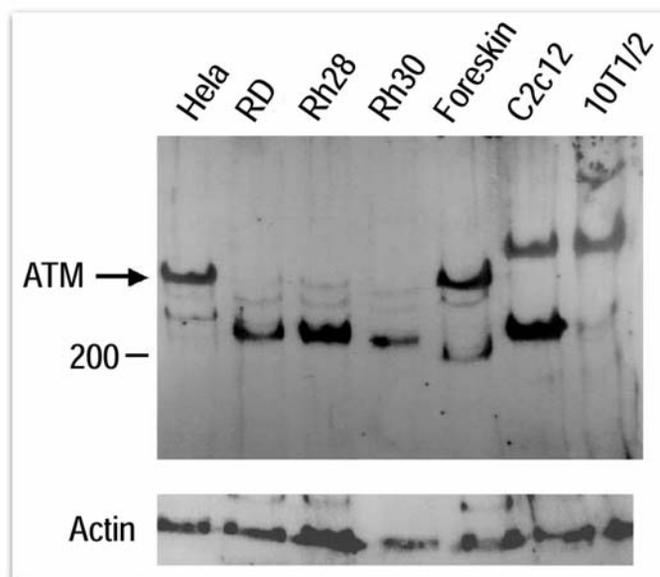

Figure 3. Western blotting of ATM protein in the cultured cells. The whole cell lysates were separated on 4.5% SDS-PAGE and the ATM protein was detected by using anti-ATM antibody. Lane 1: Hela cells; Lane 2: RD cells; Lane 3; Rh28 cells; Lane 4: Rh30 cells; Lane 5: normal human foreskin fibroblast cells; Lane 6: C2C12 mouse myoblasts; Lane 7: mouse 10T 1/2 fibroblasts.

apoptosis and DNA repair.[26-29] AT patients are known to be predisposed to a variety of cancers, and the fibroblasts of AT patients are hypersensitive to ionizing radiation.[30] Although many reports showed the association of ATM mutation with risk of a number of different human malignancies, including prostate cancer, breast cancer, ovarian cancer, B-cell chronic lymphocytic leukemia and mantle cell lymphoma, the underlying molecular mechanism by which ATM gene causes these changes are not well understood.[31-36] There is so far no study showing the association of ATM gene mutation with any solid tumor. There is enormous epidemiological interest in establishing the link of ATM gene abnormality with cancer risk, since ATM heterozygotes are estimated to represent 1.5% of total population.

We studied the immunostaining characteristics of ATM protein in clinical rhabdomyosarcoma cases and found that a significantly high number of cases lacked the proper ATM gene product (41% in 17 cases). The clinical significance of this finding is unclear at this point. There is high percentage of clinical rhabdomyosarcoma cases associated with p53 gene mutation/deletion. The p53 mutant mice tend to develop rhabdomyosarcoma.[30] P53 is a tumor suppressor regulating cell cycle control and apoptosis, and p53 is one of the many downstream targets of ATM kinase.[26,28] ATM phosphorylate p53 at Serine 15 in response to DNA damage agents.[17] It is unknown how the mutated ATM contributes to pathogenesis of rhabdomyosarcoma in the presence of the mutated p53. In fact, the rhabdomyosarcoma cells Rh30 is known to have p53 mutation, and is also positive for t(2; 13) chromosomal translocation.[11] Further investigations are needed to address these important questions.

The finding of ATM protein predominantly present in the cytoplasm, but not the nucleus of the tumor sections suggests a different role of ATM in the tumor cells. In the tumor cell lines, the ATM protein is present predominantly in the nucleus. The underlying significance of this finding is unclear. Presumably ATM protein in the cytoplasm may function differently in the tumor cells in vivo from that in the nucleus seen in the cell culture.

The murine ATM was found significantly different in size, although the ATM knockout mice recapitulate the phenotypic features of A-T patients.[37] This species difference may be important in interpreting the data from human cells and from murine cells.

References

1. Bell RS, Wunder J, Andrulis I. Molecular alterations in bone and soft-tissue sarcoma. Can J Surg 1999; 42:259-66.
2. Barr FG, Chatten J, D'Cruz CM, Wilson AE, Nauta LE, Nycum LM, et al. Molecular assays for chromosomal translocations in the diagnosis of pediatric soft tissue sarcomas. Jama 1995, 273:553-7.
3. Bridge JA, Liu J, Weibolt V, Baker KS, Perry D, Kruger R, et al. Novel genomic imbalances in embryonal rhabdomyosarcoma revealed by comparative genomic hybridization and fluorescence in situ hybridization: an intergroup rhabdomyosarcoma study. Genes Chromosomes Cancer 2000; 27:337-44.
4. Watanabe T, Sakamoto A, Tamiya S, Oda Y, Masuda K, Tsuneyoshi M. H-ras-1 point mutation in malignant peripheral nerve sheath tumors: polymerase chain reaction restriction fragment length polymorphism analysis and direct sequencing from paraffin-embedded tissues. Int J Mol Med 2000; 5:605-8.
5. Loh WE, Jr., Scrable HJ, Livanos E, Arboleda MJ, Cavenee WK, Oshimura M, Weissman BE: Human chromosome 11 contains two different growth suppressor genes for embryonal rhabdomyosarcoma. Proc Natl Acad Sci U S A 1992, 89:1755-9.
6. Barr FG, Nauta LE, Davis RJ, Schafer BW, Nycum LM, Biegel JA: In vivo amplification of the PAX3-FKHR and PAX7-FKHR fusion genes in alveolar rhabdomyosarcoma. Hum Mol Genet 1996; 5:15-21.
7. Arden KC, Anderson MJ, Finckenstein FG, Czekay S, Cavenee WK: Detection of the t(2;13) chromosomal translocation in alveolar rhabdomyosarcoma using the reverse transcriptase-polymerase chain reaction. Genes Chromosomes Cancer 1996; 16:254-60.
8. Bennicelli JL, Fredericks WJ, Wilson RB, Rauscher FJ, 3rd, Barr FG: Wild type PAX3 protein and the PAX3-FKHR fusion protein of alveolar rhabdomyosarcoma contain potent, structurally distinct transcriptional activation domains. Oncogene 1995; 11:119-30.
9. Biegel JA, Nycum LM, Valentine V, Barr FG, Shapiro DN: Detection of the t(2;13)(q35;q14) and PAX3-FKHR fusion in alveolar rhabdomyosarcoma by fluorescence in situ hybridization. Genes Chromosomes Cancer 1995; 12:186-92.
10. Fredericks WJ, Ayyanathan K, Herlyn M, Friedman JR, Rauscher FJ, 3rd: An engineered PAX3-KRAB transcriptional repressor inhibits the malignant phenotype of alveolar rhabdomyosarcoma cells harboring the endogenous PAX3-FKHR oncogene. Mol Cell Biol 2000; 20:5019-31.
11. Fredericks WJ, Galili N, Mukhopadhyay S, Rovera G, Bennicelli J, Barr FG, et al. The PAX3-FKHR fusion protein created by the t(2;13) translocation in alveolar rhabdomyosarcomas is a more potent transcriptional activator than PAX3. Mol Cell Biol 1995; 15:1522-35.
12. Savitsky K, Sfez S, Tagle DA, Ziv Y, Sartiel A, Collins FS, et al. The complete sequence of the coding region of the ATM gene reveals similarity to cell cycle regulators in different species. Hum Mol Genet 1995; 4:2025-32.
13. Savitsky K, Bar-Shira A, Gilad S, Rotman G, Ziv Y, Vanagaite L, et al. A single ataxia telangiectasia gene with a product similar to PI-3 kinase [see comments]. Science 1995; 268:1749-53.
14. Westphal CH, Rowan S, Schmaltz C, Elson A, Fisher DE, Leder P. atm and p53 cooperate in apoptosis and suppression of tumorigenesis, but not in resistance to acute radiation toxicity. Nat Genet 1997; 16:397-401
15. Westphal CH, Schmaltz C, Rowan S, Elson A, Fisher DE, Leder P. Genetic interactions between atm and p53 influence cellular proliferation and irradiation-induced cell cycle checkpoints. Cancer Res 1997; 57:1664-7.
16. Baskaran R, Wood LD, Whitaker LL, Canman CE, Morgan SE, Xu Y, et al. Ataxia telangiectasia mutant protein activates c-Abl tyrosine kinase in response to ionizing radiation [see comments]. Nature 1997; 387:516-9.
17. Canman CE, Lim DS. The role of ATM in DNA damage responses and cancer. Oncogene 1998; 17:3301-8.
18. Gately DP, Hittle JC, Chan GK, Yen TJ. Characterization of ATM expression, localization, and associated DNA-dependent protein kinase activity. Mol Biol Cell 1998; 9:2361-74.
19. Brown AL, Lee CH, Schwarz JK, Mitiku N, Piwnica-Worms H, Chung JH. A human Cds1-related kinase that functions downstream of ATM protein in the cellular response to DNA damage. Proc Natl Acad Sci USA 1999; 96:3745-50.
20. Tominaga K, Morisaki H, Kaneko Y, Fujimoto A, Tanaka T, Ohtsubo M, et al. Role of human Cds1 (Chk2) kinase in DNA damage checkpoint and its regulation by p53. J Biol Chem 1999; 274:31463-7.
21. Zhao S, Weng YC, Yuan SS, Lin YT, Hsu HC, Lin SC, et al. Functional link between ataxia-telangiectasia and Nijmegen breakage syndrome gene products [see comments]. Nature 2000; 405:473-7.
22. Shiloh Y. Ataxia-telangiectasia and the Nijmegen breakage syndrome: related disorders but genes apart. Annu Rev Genet 1997; 31:635-62.
23. Wu X, Ranganathan V, Weisman DS, Heine WF, Ciccone DN, O'Neill TB, et al. ATM phosphorylation of Nijmegen breakage syndrome protein is required in a DNA damage response [see comments]. Nature 2000; 405:477-82.
24. Puri PL, Wu Z, Zhang P, Wood LD, Bhakta KS, Han J, et al. Induction of terminal differentiation by constitutive activation of p38 MAP kinase in human rhabdomyosarcoma cells. Genes Dev 2000, 14:574-84.
25. Schaffner C, Idler I, Stilgenbauer S, Dohner H, Lichter P. Mantle cell lymphoma is characterized by inactivation of the ATM gene. Proc Natl Acad Sci USA 2000; 97:2773-8.
26. Wang JY. Cellular responses to DNA damage. Curr Opin Cell Biol 1998, 10:240-7.
27. Wang JY: Regulation of cell death by the Abl tyrosine kinase. Oncogene 2000; 19:5643-50.
28. Kastan MB. Lim DS. The many substrates and functions of ATM. Nat Rev Mol Cell Biol 2000; 1:179-86.
30. Kastan MB. Cell cycle. Checking two steps. Nature 2001; 410:766-7.
31. Anderson J, Gordon A, Pritchard-Jones K, Shipley J. Genes, chromosomes, and rhabdomyosarcoma. Genes Chromosomes Cancer 1999; 26:275-85.
32. Fan Z, Chakravarty P, Alfieri A, Pandita TK, Vikram B, Guha C. Adenovirus-mediated antisense ATM gene transfer sensitizes prostate cancer cells to radiation [In Process Citation]. Cancer Gene Ther 2000; 7:1307-14.
33. Angele S, Hall J. The ATM gene and breast cancer: is it really a risk factor? Mutat Res 2000; 462:167-78.
34. Buchholz TA, Weil MM, Story MD, Strom EA, Brock WA, McNeese MD. Tumor suppressor genes and breast cancer. Radiat Oncol Investig 1999; 7:55-65.
35. Capello D, Gaidano G. Molecular pathophysiology of indolent lymphoma [see comments]. Haematologica 2000; 85:195-201.
36. Koike M, Takeuchi S, Park S, Hatta Y, Yokota J, Tsuruoka N, et al. Ovarian cancer: loss of heterozygosity frequently occurs in the ATM gene, but structural alterations do not occur in this gene. Oncology 1999; 56:160-3.
37. Schaffner C, Stilgenbauer S, Rappold GA, Dohner H, Lichter P. Somatic ATM mutations indicate a pathogenic role of ATM in B-cell chronic lymphocytic leukemia. Blood 1999; 94:748-53.
38. Xu Y, Ashley T, Brainerd EE, Bronson RT, Meyn MS, Baltimore D. Targeted disruption of ATM leads to growth retardation, chromosomal fragmentation during meiosis, immune defects, and thymic lymphoma [see comments]. Genes Dev 1996; 10:2411-22.