ATM-dependent expression of the insulin-like growth factor-I receptor in a pathway regulating radiation response

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The ATM gene is mutated in the syndrome of ataxia telangiectasia (AT), associated with neurologic dysfunction, growth abnormalities, and extreme radiosensitivity. Insulin-like growth factor-I receptor (IGF-IR) is a cell surface receptor with tyrosine kinase activity that can mediate mitogenesis, cell transformation, and inhibition of apoptosis. We report here that AT cells express low levels of IGF-IR and show decreased IGF-IR promoter activity compared with wild-type cells. Complementation of AT cells with the ATM cDNA results in increased IGF-IR promoter activity and elevated IGF-IR levels, whereas expression in wild-type cells of a dominant negative fragment of ATM specifically reduces IGF-IR expression, results consistent with a role for ATM in regulating IGF-IR expression at the level of transcription. When expression of IGF-IR cDNA is forced in AT cells via a heterologous viral promoter, near normal radioresistance is conferred on the cells. Conversely, in ATM cells complemented with the ATM cDNA, specific inhibition of the IGF-IR pathway prevents correction of the radiosensitivity. Taken together, these results establish a fundamental link between ATM function and IGF-IR expression and suggest that reduced expression of IGF-IR contributes to the radiosensitivity of AT cells. In addition, because IGF-I plays a major role in human growth and metabolism and serves as a survival and differentiation factor for developing neuronal tissue, these results may provide a basis for understanding other aspects of the AT syndrome, including the growth abnormalities, insulin resistance, and neurodegeneration.

The insulin-like growth factor-I receptor (IGF-IR) is a tyrosine kinase, transmembrane receptor expressed in almost all body tissues. IGF-IR is an important regulator of cell growth and differentiation, transformation, and apoptosis (1), and it is found to be overexpressed in some human cancers (2). Targeted disruption of the IGF-IR in knockout mice results in animals weighing 45% less than normal, and these animals die at birth from multiple organ abnormalities (3). The ability of the IGF-IR to protect cells from apoptotic injuries, including ionizing radiation (IR), is well documented (4, 5). The mitogenic and survival signals originating from the IGF-IR use at least three pathways, one of which is dependent on phosphatidylinositol 3-kinase activity (6, 7).

To probe cellular regulation of the IGF-IR pathway, we investigated IGF-IR expression in a variety of cell lines with defined genetic backgrounds. These studies included an examination of cells derived from individuals with the syndrome ataxia telangiectasia (AT), an autosomal recessive human genetic disorder with a pleiotropic phenotype including neurodegeneration, immunodeficiency, growth abnormalities, premature aging, and radiosensitivity (8, 9). Cells with mutations in the AT gene, designated ATM, exhibit marked sensitivity to ionizing radiation and show abnormalities in cell cycle regulation and DNA metabolism (10). The ATM protein is thought to function in signal transduction and belongs to a family of lipid and protein kinases based on homology to phosphatidylinositol 3-kinase (9, 11).

Here, we report that ATM cells show low levels of IGF-IR expression and that this deficiency can be corrected by complementation with the wild-type ATM cDNA. Inhibition of ATM function in wild-type cells results in decreased receptor expression, and experiments with reporter constructs indicate that ATM regulates IGF-IR at the level of transcription. Forced expression of IGF-IR in AT cells confers increased radioresistance, and blocking IGF-IR function in ATM-corrected cells causes radiosensitivity. These results suggest that ATM has a fundamental role in the regulation of IGF-IR and that the IGF-IR pathway has a major influence on the radiosensitivity of AT cells.

Materials and Methods

Cells. Cells were obtained from the Coriell Institute for Medical Research (Camden, NJ). GM5849, GM9607, GM24, and GM637 are SV40-transformed fibroblast cell lines, with GM5849 and GM9607 obtained from AT-affected individuals and GM24 and GM637 obtained from apparently normal individuals. GM3487 and GM3489 are primary human fibroblasts obtained from an AT-affected individual and from his heterozygous parent, respectively. All cells were grown in DMEM supplemented with 10% FBS and 5% penicillin-streptomycin solution (all from Life Technologies, Rockville, MD), unless otherwise stated.

Western Blot Analysis. For Western blot analysis, 5×10^5 cells were plated in 60-mm dishes and grown to 70% confluence. Cells were washed with PBS and then scraped into RIPA buffer (PBS with 1% Nonidet P-40/0.5% sodium deoxycholate/0.1% SDS) with aprotinin (0.02 mg/ml), sodium orthovanidate (1 mM), and phenylmethylsulfonyl fluoride (0.1 mg/ml). Protein concentration was measured using a BCA protein assay (Pierce), and equal amounts of protein were mixed with sample buffer and boiled for 5 min. The samples were loaded and separated on an 8% polyacrylamide gel. Proteins were transferred by electroblotting onto a polyvinylidene fluoride membrane and probed using 1 μg/ml IGF-IR β subunit antibody or epidermal growth factor receptor (EGFR) antibody (sc-713 and sc-03, respectively, 1:200 dilution, Santa Cruz Biotechnology) in the presence of TBS/0.1% Tween 20/5% nonfat dry milk. The secondary antibody used was anti-rabbit IgG-horseradish peroxidase (sc-2004, Santa Cruz Biotechnology). Proteins were detected using ECL Western blotting procedure (Amersham Pharmacia) following the manufacturer's instructions. Actin expression was also assayed to

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Abbreviations: AT, ataxia telangiectasia; EGFR, epidermal growth factor receptor; IGF-IR, insulin-like growth factor-I receptor; LZ, leucine zipper; IR, ionizing radiation.

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confirm that protein loading was equal for all samples (data not shown).

ATM levels were examined by combined immune precipitation and Western blot analysis. Cell lysates were collected, and 100 μg of each lysate was incubated with 5 μl of ATM antibody (H-248, sc-7230, Santa Cruz Biotechnology) and allowed to complex for 1 h. Twenty microliters of Protein G Plus-Agarose conjugate (Santa Cruz Biotechnology) was added, and after a 1-h incubation period, the complex was washed four times with RIPA buffer and boiled for 5 min. The proteins were separated on an 8% polyacrylamide gel. Proteins were electroblotted onto a nitrocellulose membrane and detected by probing with 1 μg/ml ATM antibody in TBS with 0.1% Tween 20/5% nonfat dry milk. The secondary antibody used was anti-rabbit IgG-horseradish peroxidase (Santa Cruz Biotechnology). Proteins were detected using ECL Western blotting procedure (Amersham Pharmacia) following the manufacturer's instructions.

Transfections. GM5849 (AT mutant) cells were cotransfected with a vector expressing wild-type ATM cDNA (pcDNA3 flag-ATM) provided by M. Kastan (St. Jude Children's Research Hospital, Memphis, TN) along with a plasmid expressing the hygromycin resistance gene, pWE4, obtained from the American Type Culture Collection (ATCC), at a mass ratio of 10:1. Control cells were transfected only with hygromycin resistance plasmid. Transfection was carried out using the cationic lipid formulation, Fugene 6 (Boehringer Mannheim), as directed by the manufacturer. Cells were selected in medium containing 400 μg/ml hygromycin, and surviving colonies were isolated and expanded after 2 weeks. Resulting colonies were maintained in growth medium supplemented with 100 μg/ml hygromycin.

GM637 cells were transfected with a vector expressing the FB2F cDNA fragment of ATM containing the leucine zipper domain subcloned into pcDNA3, provided by S. Powell (Massachusetts General Hospital, Boston, MA). This plasmid contains a neomycin resistance gene. Control cells were transfected with an empty pcDNA3 vector. Cells were selected in medium containing 400 μg/ml neomycin, and colonies were isolated and expanded after 2 weeks. Resulting colonies were maintained in growth medium supplemented with 400 μg/ml neomycin.

GM5849 cells, an SV40-transformed fibroblast line from an AT individual, were cotransfected with the vector pCVNIGF-IR (expressing the human IGF-IR cDNA driven by the SV40 promoter) along with pWE4 carrying the hygromycin resistance gene (at a 1:10 ratio). Control cells were transfected with an empty pCVN vector. Transfected clones were selected, isolated, and grown as above.

Clonogenic Survival Assays. Following the method of Huo *et al.* (12), cells were seeded in 96-well dishes at 15 or 30 cells per well. After adhering to the plates, cells were exposed to IR using a Cs-137 irradiator source at a dose rate of 225 rads/min, and viable colonies were stained after 10 days. Colony-forming efficiency was calculated as $(-\ln F)/W$, where F is fraction of negative wells and W is the number of cells seeded per well. Survival fraction was determined as CFE (IR plate)/CFE (control plate) $\times$ 100. The average survival and standard deviation from at least three experiments are presented.

Promoter Activation Assays. The IGF-IR promoter-luciferase reporter plasmid, provided by D. LeRoith (National Institutes of Health, Bethesda, MD), contains the promoter of the rat IGF-IR gene fused to a promoterless luciferase reporter gene in the pOLUC vector (13). The control β-galactosidase vector (pSVβgal) was obtained from Promega and carries the lacZ gene coding region under the control of the SV40 promoter. Cells were plated at a density of 2×10^5 cells in 60-mm dishes and allowed to adhere for at least 4 h. Cells were then transfected with a plasmid containing the luciferase gene driven by the IGF-IR promoter, along with the β-galactosidase expressing vector as a control for transfection. Transfection was carried out using the cationic lipid formulation, Fugene 6 (Boehringer Mannheim) as directed by the manufacturer. Twenty-four hours after transfection, cell extracts were prepared using reporter lysis buffer (Promega). Luciferase (luciferase assay system, Promega) or β-gal (Galacto-Star, Tropix, Bedford, MA) activity was measured using a luminometer. The luciferase activity, as a measure of IGF-IR promoter activation, was normalized to β-gal expression to adjust for variations in transfection efficiency. The average relative luciferase expression and standard deviation from at least three experiments are shown.

Antibody-Mediated Inhibition of IGF-IR Function. 5849cA4 cells, a subclone of the AT-deficient cell line, GM5849, transfected with the wild-type ATM cDNA, were seeded into 96-well plates at 15 or 30 cells per well in either normal media or media supplemented with a 1:200 dilution of anti-IGF-IR antibody (Ab-1, Oncogene Research Products, Cambridge, MA), followed by exposure to IR. Colony formation and survival were determined as above.

Dominant Negative Inhibition of IGF-IR Function. 5849cA4 cells, a subclone of the AT-deficient cell line, GM5849, transfected with the wild-type ATM cDNA, were further transfected with a vector, pCVNIGF-IRSolRec, expressing a truncated, dominant negative variant of IGF-IR, 486 stop (SolRec). This plasmid contains a neomycin resistance gene for selection. Cells were selected, isolated, and grown as above. Control cells were transfected with an empty pCVN vector.

Results

ATM Regulates IGF-IR Expression. A series of unrelated wild-type and AT mutant cell lines and a pair of primary cell cultures from an affected AT individual and his heterozygous parent (GM3487 and GM3489, respectively) were assayed by Western blot for IGF-IR expression levels (Fig. 1*A*). The AT cells were consistently found to have lower steady-state levels of IGF-IR protein than either the heterozygous or wild-type ones. Because these initial results were obtained in non-isogenic cell lines, we sought to more directly test the role of ATM in IGF-IR expression by establishing a series of otherwise isogenic cell lines differing only in ATM status. We transfected one AT mutant line, 5849, with a vector expressing the full-length ATM cDNA (14). Eight 5849 subclones were identified as successful transfectants by growth in the presence of hygromycin selection, and new expression of ATM protein was confirmed by immune precipitation and Western blot analysis (Fig. 1*B*). To determine if the complementation of the ATM cells with the ATM cDNA had resulted in elevated IGF-IR expression levels, a Western blot analysis was performed in parallel on proteins from the same cells using an anti-IGF-IR antibody (Fig. 1*B*). All of the subclones expressing the wild-type ATM were also found to express increased levels of IGF-IR compared with the parental AT mutant cells, consistent with a role for ATM in regulating IGF-IR expression.

In two of the 5849 subclones transfected with ATM cDNA, the functional correction of the ATM phenotype was confirmed by determining radiation survival in a clonogenic assay (12). The two subclones tested, 5849cA2 and 5849cA4, showed substantial correction of the parental radiosensitivity (Fig. 1*C*), although not exactly to the level of radioresistance of the unrelated wild-type cell line (GM637).

To determine if ATM specifically modulates IGF-IR, or if it is a general regulator of transcription, cells with and without ATM were assayed by Western blot for expression of the insulin receptor, the EGFR, and fibroblast growth factor receptor. The levels of insulin receptor, EGFR, and fibroblast growth factor

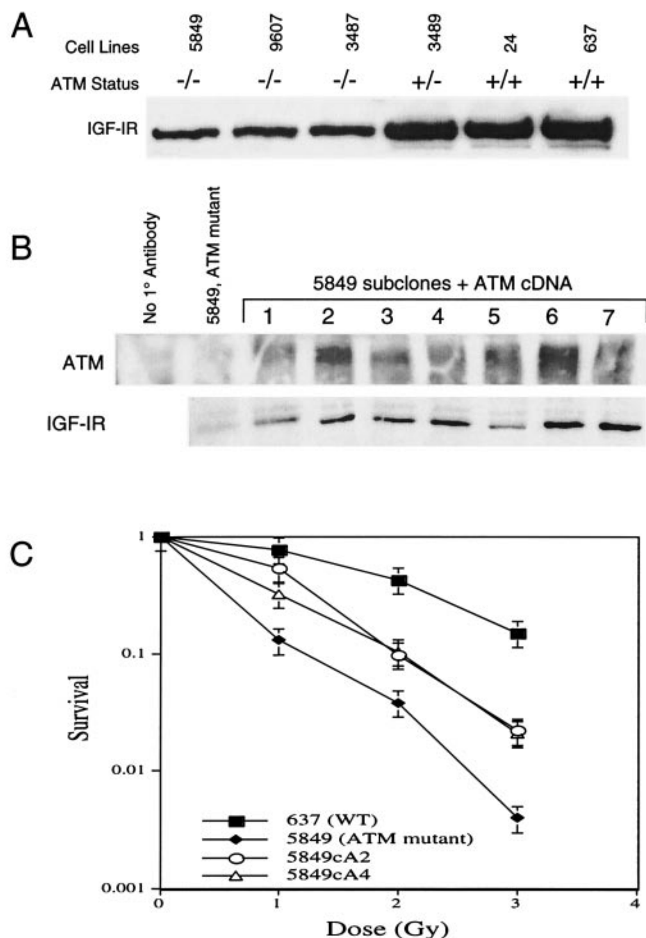

Fig. 1. Influence of ATM on IGF-IR expression. (*A*) Western blot analysis of IGF-IR expression levels in AT mutant, heterozygous, and wild-type human cells. All cells are SV40-transformed fibroblasts, except 3487 and 3489, which are primary fibroblasts from an affected individual and his heterozygous parent. (*B*) Analysis of ATM and IGF-IR expression in GM5849 cells, an SV40-transformed human fibroblast cell line from an AT individual, and a series of subclones transfected with wild-type ATM cDNA. ATM expression was determined by immune precipitation with anti-ATM antibody, followed by Western blot analysis with the same antibody. IGF-IR expression was determined directly by Western blot. (*C*) Confirmation of functional complementation of GM5849 cells by ATM cDNA expression based on radiation response. Clonogenic survival was determined following IR in the ATM-deficient GM5489 cells and in two subclones (5849cA2 and 5849cA4), transfected with a vector expressing the wild-type ATM cDNA. Also included for comparison is an unrelated wild-type cell line, GM637.

receptor appeared to be the same regardless of ATM status (data not shown).

IGF-IR Promoter Activity Is Reduced in AT Cells. To elucidate the mechanism for the increased IGF-IR expression, the 5849 ATM cells and one of the subclones complemented with the ATM cDNA (subclone 5849cA4) were assayed for their ability to activate the IGF-IR promoter. The cells were transfected with a construct consisting of the IGF-IR promoter driving a luciferase reporter gene (13), in combination with a vector carrying the β-galactosidase gene driven by the SV40 promoter as a control. After normalization to β-galactosidase levels, the ATM cDNA-corrected cells showed 4-fold higher levels of IGF-IR promoter activation compared with the parental ATM mutant cells (Fig. 2*A*).

Additional evidence for altered IGF-IR promoter activity based on ATM function was obtained in luciferase expression experiments using the IGF-IR promoter-luciferase construct in primary fibroblasts from an ATM-affected individual (GM3487) and his heterozygous parent (GM3489). This comparison shows, again, that IGF-IR promoter activity is reduced in AT mutant cells (Fig. 2*B*). In addition, this analysis demonstrates that reduced IGF-IR promoter activity is found not only in established, SV40-transformed AT cell lines but also in AT primary cells, ruling out the possibility that this effect of ATM could be unique to SV40-transformed cell lines.

Furthermore, multiple studies performed with (*i*) an ATM mutant cell line versus otherwise isogenic, ATM cDNA-corrected subclones and (*ii*) a number of related and unrelated ATM mutant, heterozygous, and wild-type cell lines and primary cells indicate a link between ATM function and IGF-IR expression. The IGF-IR immunoblot analyses demonstrate reduced IGF-IR levels in all of the ATM-deficient cells tested, and the promoter activation measurements indicate that the reduced receptor levels can be attributed to subnormal IGF-IR promoter activity. The consistent results found in multiple cell lines of varied origin argue against the findings being a peculiar characteristic of one cell line and support the concept that there is a fundamental role for ATM in regulating IGF-IR expression.

Inhibition of ATM Decreases IGF-IR Levels. To further verify this connection between ATM and IGF-IR, a wild-type human fibroblast cell line, 637, was transfected with a vector expressing a fragment of the ATM protein containing the leucine zipper (LZ) domain. This fragment has been shown previously to manifest dominant negative activity with respect to radiosensitivity, but not with respect to the cell cycle checkpoint functions associated with ATM (14). Six subclones of the 637 line derived by transfection with the LZ domain construct were assayed for IGF-IR expression by Western blot, and all were found to express lower levels of IGF-IR than the parental 637 line (Fig. 3*A*). These same subclones were assayed for the expression of EGFR, and no difference was found compared with the parental cells, suggesting that the LZ domain specifically affects levels of IGF-IR (Fig. 3*A*). Two of these subclones (LucZip1 and LucZip4) were also tested for clonogenic survival following IR, and both were found to be relatively radiosensitive compared with the parental 637 cells (Fig. 3*B*), demonstrating successful expression of a dominant negative phenotype [in keeping with the data of Morgan *et al.* (14)] and establishing a correlation of reduced IGF-IR expression levels with radiosensitivity in the setting of altered ATM function.

Forced Expression of IGF-IR in AT Cells Results in Increased Cell Survival Following IR. Based on the above results and on studies showing that IGF-IR has an important role in regulating cellular apoptotic responses to selected cytotoxic agents, including ionizing radiation (4, 5), we hypothesized that the below-normal levels of IGF-IR in AT cells might be a critical factor contributing to the radiosensitivity of the cells. To directly test the significance of IGF-IR levels in the radiation response of AT cells, we experimentally manipulated IGF-IR expression by transfection of the 5849 AT cells with a vector expressing the full-length IGF-IR cDNA driven by a heterologous SV40 promoter (5, 15). Five transfected subclones were isolated by growth in selective medium. IGF-IR expression was assayed by Western blot (Fig. 4*A*), and all five showed increased IGF-IR expression to varying levels. Two of these, subclones 3 and 7 in Fig. 4*A* (designated 5849cI3 and 5849cI7, respectively, in Fig. 4*B*), were chosen for analysis, and clonogenic cell survival assays in response to ionizing radiation were carried out (Fig. 4*B*). Both of these AT cell subclones with forced expression of IGF-IR were found to have increased survival after ionizing radiation, with a level of radioresistance approaching that of an unrelated wild-

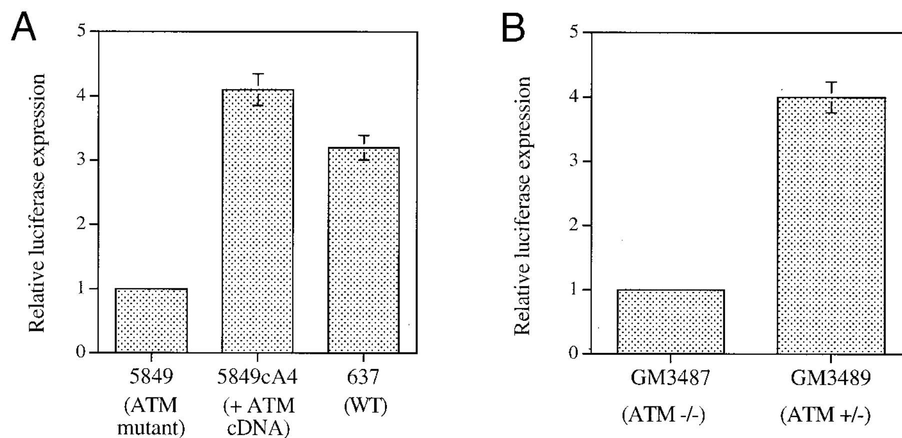

Fig. 2. ATM-dependent activation of the IGF-IR promoter. To examine activation of the IGF-IR promoter as a function of ATM status, the indicated cells were transfected with a plasmid containing the luciferase gene driven by base pairs −2350 to +640 of the rat IGF-IR promoter region, along with a β-galactosidase expression vector as a control for transfection efficiency. The luciferase activity, as a measure of IGF-IR activation, was normalized to β-gal expression in each case. Experiments were performed in triplicate, and the results shown are the mean of three separate experiments. (*A*) Comparison of IGF-IR promoter activity in ATM-deficient GM5849 cells (SV40-transformed fibroblasts) and a subclone transfected with a vector expressing wild-type ATM cDNA (5849cA2), along with an unrelated wild-type SV40 transformed fibroblast cell line, GM637. (*B*) Comparison of IGF-IR promoter activity in primary AT fibroblasts obtained from an affected individual (GM3487) and from his heterozygous parent (GM3489).

type cell line (637). Interestingly, the extent of radioresistance conferred by forced expression of IGF-IR was similar to that achieved by complementation of the same parental cells with the ATM cDNA (compare Figs. 1*C* and 4*B*).

Inhibition of IGF-IR Reduces Clonogenic Cell Survival Following IR in ATM Cells Complemented with ATM cDNA. To further test the importance of IGF-IR in the radiation response of ATM cells, we used strategies to specifically inhibit the IGF-IR pathway in ATM cells that had otherwise been "corrected" by complementation with the ATM cDNA. 5849 subclone designated 5849cA4, shown to express the transfected ATM cDNA, to have increased IGF-IR levels, and to have increased radiation survival compared with the parental 5849 cells (Fig. 1 *B* and *C*), was tested for clonogenic survival after IR in the presence of medium containing a neutralizing anti-IGF-IR antibody directed against the extracellular domain. This anti-IGF-IR antibody treatment resulted in reduced clonogenic survival of the cells following ionizing radiation, approaching that of the original 5489 AT mutant cells (Fig. 5*A*).

In a separate experiment, as another means to block IGF-IR function, the 5849cA4 cells were transfected with a vector expressing a truncated variant of IGF-IR (486stop) that has been shown to have dominant negative activity (16, 17). Stable subclones were established by selection in zeocin, and one was tested for radiation survival (Fig. 5*B*). In addition, a number of subclones arising after zeocin selection were pooled, and the pooled cells were also assayed for clonogenic survival (Fig. 5*B*). Both the individual subclone and the pooled subclones showed reduced IR survival compared with the 5849cA4 line that had otherwise been corrected with the ATM cDNA. Thus, inhibition of IGF-IR by two different methods provides evidence that the IGF-IR pathway contributes to the ability of the wild-type ATM gene to confer a normal radiation response, as measured by clonogenic survival.

Discussion

The data reported here show that ATM plays an important regulatory role in IGF-IR expression. AT mutant cells have low levels of IGF-IR, and complementation with the ATM cDNA results in elevated IGF-IR levels. In addition, AT cells show minimal activation of the IGF-IR promoter, a deficiency cor-

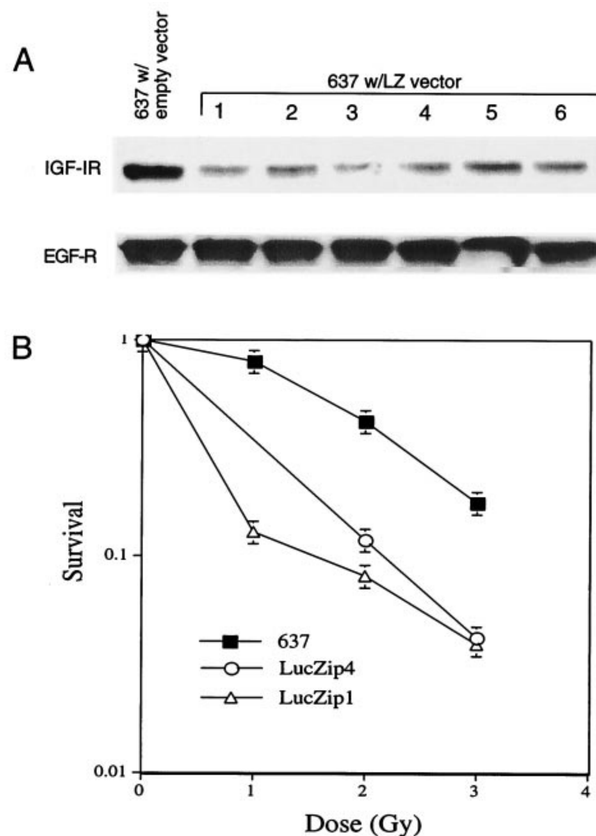

Fig. 3. Effect of expression in wild-type cells of a dominant negative ATM fragment on (*A*) IGF-IR and EGFR levels and (*B*) clonogenic survival following IR. An SV40-transformed fibroblast cell line from an apparently normal individual (GM637) was transfected with a vector expressing a fragment of ATM containing the leucine zipper domain, which has been shown to function as a dominant negative protein (14). IGF-IR and EGFR levels were determined by Western blot in the 637 cells and in the cells transfected with the dominant negative leucine zipper fragment. Subclones 1 and 4 (LucZip1 and LucZip4) were chosen for analysis of radiation survival in comparison with the parental 637 cells.

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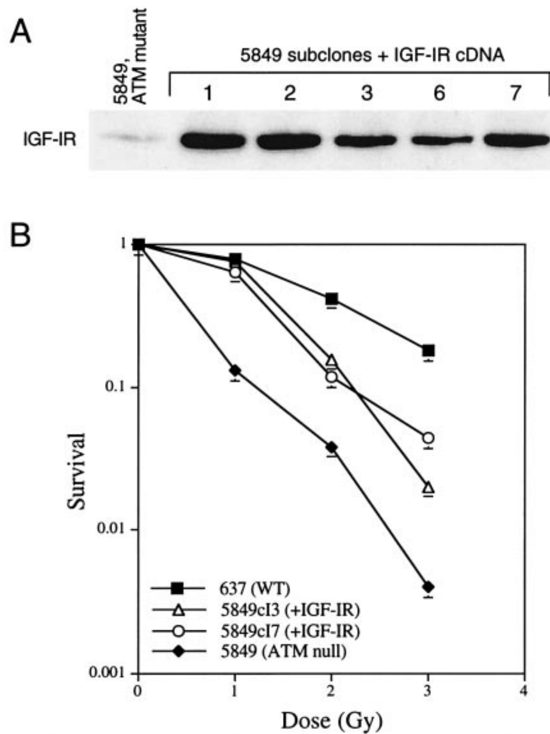

Fig. 4. Increased radiation survival in AT cells upon forced expression of IGF-IR. (*A*) Western blot analysis of IGF-IR expression in GM5849 cells, which lack ATM, and in GM5849 subclones transfected with a plasmid-expressing IGF-IR driven by a viral promoter. (*B*) Clonogenic survival after exposure to IR of subclones 3 (5849cl3) and 7 (5849cl7), both of which were judged by Western blot in *A* to express increased levels of IGF-IR, in comparison with the parental 5849 AT mutant cells and to an unrelated wild-type cell line, 637.

rected by ATM cDNA complementation. This latter observation indicates that the defect in IGF-IR expression in AT cells is at least partly at the level of transcription. However, the exact pathway(s) by which ATM influences IGF-IR promoter activity remains to be determined. Factors proposed to be downstream of ATM that could mediate effects on gene expression include p53, c-abl, NF-κB, and SAPK (18–23). Intriguingly, p53 has been shown to influence IGF-IR promoter activity, as have WT1 and SP1 (24–26). However, other mechanisms could lead to abnormal receptor levels and cannot be ruled out as playing some additional role. For example, Lim *et al.* have suggested a role for ATM in cell surface receptor endocytosis (27).

It should be noted that the effect of ATM on IGF-IR levels reported here is not a general one affecting other cell surface receptors. We compared by Western blot the levels of the insulin receptor, EGFR, and fibroblast growth factor receptor in the 5849 AT mutant cells with the levels in the 5849cA2 and 5849cA4 subclones complemented with the ATM cDNA. No significant differences were seen (data not shown). In contrast, a substantial difference was detected in IGF-IR levels depending on ATM status (Fig. 1 *A* and *B*). Furthermore, stably transfecting cells with the dominant negative LZ domain of ATM results in a decrease in IGF-IR levels but no change in EGFR levels (Fig. 3*A*).

The ability of the dominant negative LZ domain of ATM to cause a reduction in IGF-IR levels in wild-type cells further supports the association of ATM function with IGF-IR expression. Interestingly, the dominant negative phenotype of the LZ domain has been shown to include just a subset of the cellular abnormalities associated with AT (14). Morgan *et al.* (14) found that expression of the LZ domain in wild-type cells does not produce the cell cycle checkpoint abnormalities seen in AT cells, but it does cause the radiosensitivity. Our results confirm their finding that the dominant negative LZ domain confers radiosensitivity and provide a specific correlation of this radiosensitivity effect with reduced IGF-IR levels.

The forced expression of IGF-IR in AT cells was found to confer increased radioresistance, showing directly that increased IGF-IR expression in AT cells is sufficient to reverse the radiosensitivity. However, increasing IGF-IR does not fully complement the radiation sensitivity of the AT cells up to that of wild-type cells. This is probably because IGF-IR is only one of a number of factors downstream of ATM that can affect the response of cells to radiation. The effect of IGF-IR on radiation

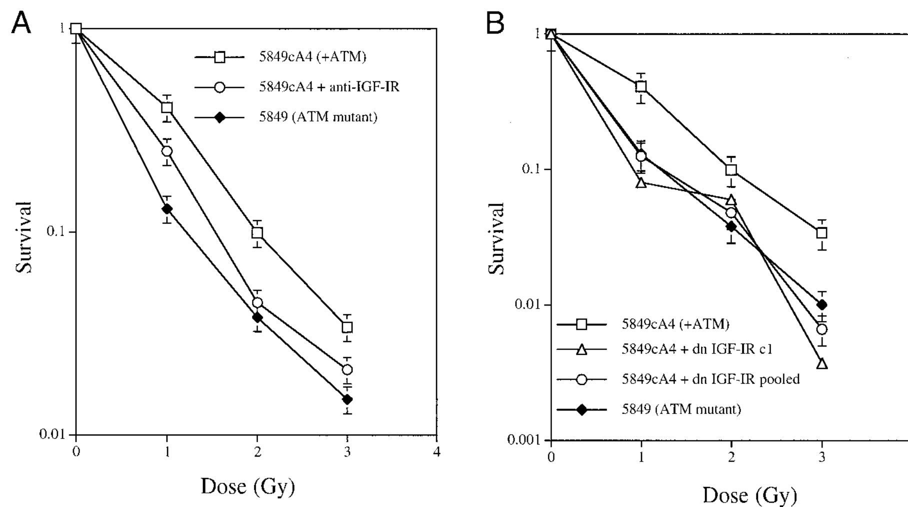

Fig. 5. Specific inhibition of the IGF-IR pathway blocks correction of the radiosensitivity in AT cells by the ATM cDNA. GM5849 cells, deficient in ATM, were transfected with an ATM expression plasmid, yielding subclones expressing wild-type ATM (see Fig. 1). (*A*) The radiation survival of one complemented subclone, 5849cA4, was determined in the presence and absence of a neutralizing anti-IGF-IR antibody added to the growth medium. (*B*) The ATM cDNA-complemented subclone 5849cA4 was further transfected with a vector expressing a dominant negative variant of IGF-IR (486stop). The clonogenic survival of one transfectant subclone (c1) and of a cell population consisting of a number of pooled transfected clones (pooled) was determined in comparison with 5849cA4 and with the original parent line, 5849.

response is consistent with the anti-apoptotic and pro-survival function of IGF-IR (4) and with our previous observation that IGF-IR overexpression in otherwise wild-type cells can render cells relatively radioresistant (5).

We found that blocking the IGF-IR pathway using either an anti-IGF-IR antibody or a dominant negative IGF-IR variant resulted in reduced radiation survival in AT cells that had otherwise been complemented with the ATM cDNA. This finding suggests that the IGF-IR pathway plays an important role in the restoration of a normal radiation response in AT cells by wild-type ATM. Hence, the abnormal IGF-IR expression in AT cells appears to contribute to the extreme radiosensitivity of the cells. This consequence of reduced IGF-IR expression in AT cells can be attributed to the well established role of the IGF-IR in promoting cell survival under stress (4, 28). Therefore, in wild-type cells where normal ATM function results in increased IGF-IR expression, the ATM/IGF-IR axis may set a higher tolerance in response to cell stress, including IR.

In early attempts to clone the ATM gene, a number of non-ATM cDNAs with the ability to complement some aspect of the damage response phenotype were identified (9, 29). The ability of forced IGF-IR expression to confer radioresistance would seem to place it in this class. However, the direct connection between ATM function and IGF-IR expression provides strong evidence that IGF-IR has a fundamental role in mediating the effects of ATM and that the phenotype of radiosensitivity in AT cells is partly related to the abnormal expression of IGF-IR.

Besides playing a role in the radiosensitivity of AT, the reduced IGF-IR levels in AT cells may have other manifestations both in the cellular phenotype and in the human syndrome. For example, AT cells are known to grow poorly in culture and have increased requirements for serum growth factors (30–35). This could reflect the low levels of IGF-IR in these cells. AT individuals suffer a variety of growth abnormalities and, in some cases, have been reported to exhibit insulin resistance (9, 36). Because of the physiologic role of IGF-I in human growth and metabolism, ATM-associated abnormalities in the IGF-IR pathway could provide a basis for understanding these observations. In addition, IGF-IR has a role in the developing brain and has been shown to function as a survival and differentiation factor for neuronal cells (37–39). Hence, decreased IGF-IR expression in AT cells could be a factor contributing to the neurodegeneration seen in affected individuals.

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