Loss of ATM Activates Rac1 and Alters Cell Migration

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Abstract

Ataxia-telangiectasia (A-T) is a neurodegenerative disease characterized by motor impairments and a predisposition to metastatic cancer. A-T is caused by a mutation in the serine-threonine kinase ataxia-telangiectasia mutated (ATM). ATM is integral in the response to DNA double-stranded breaks (DSBs) as well as the control of cellular redox status. The Rho family of small GTPases including the subfamily member, Rac1 play important regulatory roles in cellular growth, adhesion, motility, and cancer formation. Rac1 is regulated by three classes of proteins: guanine-nucleotide exchange factors (GEFs) promote the active state by facilitating GTP binding, GTPase-activating proteins (GAPs) suppress their activity by accelerating GTP hydrolysis, and Rho GDP-dissociation inhibitors (GDI) sequester inactive GDP-bound Rac1 in the cytosol. Recent studies have also shown that Rac1 can be activated directly by reactive oxygen species (ROS) in a GEF-independent pathway. However, the connection between ATM and the regulation of Rac1 has not been extensively examined. Previous research from our lab has shown that loss of ATM activates Rac1 via a ROS-mediated mechanism. We therefore sought to determine the consequences of activated Rac1 in cells lacking ATM. Using human cell lines proficient and deficient in ATM, we monitored the effects on cellular migration using single cell bioimaging techniques. Interestingly, cells that lacked functional ATM not only displayed activated Rac1 but also increases in cell migration in contrast to ATM wild type cells. Understanding the mechanisms of ATM-mediated Rac1 activity may clarify why patients with A-T have a predisposition to metastatic cancer.

Methods

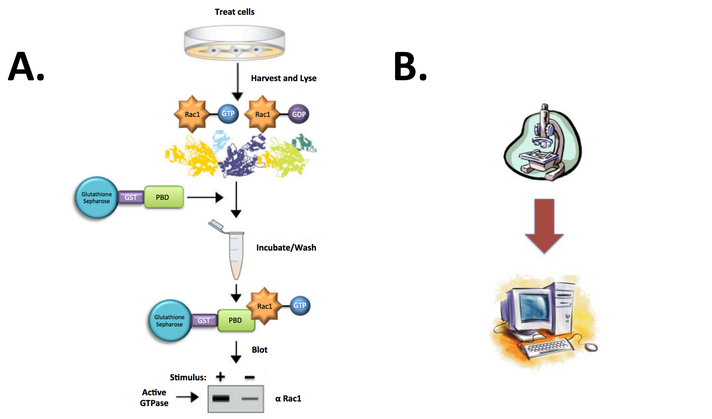


Figure 1: Experimental methods. **(A)** The p21 binding domain (PBD) pull-down is a immunoprecipitation-based assay that measures active Rac1 levels by binding to GTP bound Rac1. **(B)** The migration of ATM-proficient and -deficient cells were measured every 10 minutes over 16 h with a Nikon BioStation IM (20X Objective). Cell velocity, accumulated distance, and directionality were measured with NIH Image J software using the manual tracking plug-in.

Inhibition of ATM Increases Activated Rac1

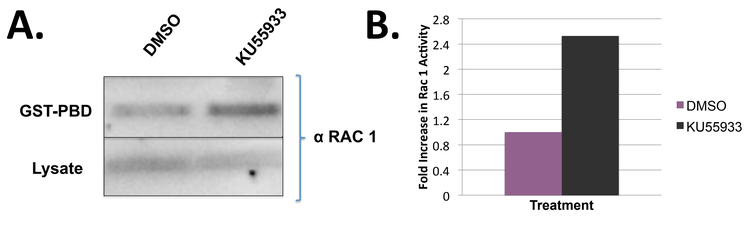


Figure 2: ATM inhibition increases activated Rac1. **(A)** Western blot image showing activated Rac1 (Rac1-GTP) in HeLa cells treated with DMSO or KU55933, an ATM inhibitor. **(B)** Quantification of activated Rac1 in HeLa cells treated with DMSO or KU55933. Data shown is from a single representative western blot image analyzed with NIH Image J software.

ATM Status Does Not Affect Cell Directionality

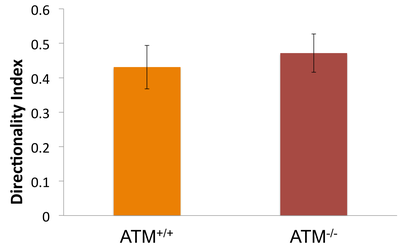


Figure 2: Directionality in ATM-deficient cells is unaltered compared to ATM-proficient cells. Fibroblasts were plated on fibronectin coated coverslips and imaged every 10 minutes over 16 h with a Nikon BioStation IM (20X Objective). The average directionality (1-persistant, 0-random) was measured with NIH Image J software using the manual tracking plug-in. Error bars represent the S.E.M.

ATM Deficiency Increases Cell Velocity

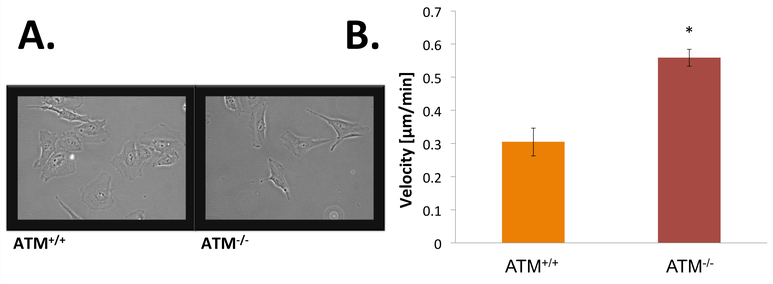


Figure 3: Velocity in ATM-deficient cells increases compared to ATM-proficient cells. **(A)** Still-images of ATM-proficient and ATM-deficient firoblasts. Fibroblasts were plated on fibronectin coated coverslips and imaged every 10 minutes over 16 h with a Nikon BioStation IM (20X Objective). **(B)** Average cell velocity (µm/min). Cell velocity was measured with NIH Image J software using the manual tracking plug-in. Error bars represent the S.E.M. and the student's *t*-test for paired samples was used to determine * $p < 0.01$.

ATM Deficiency Increases Cell Migration

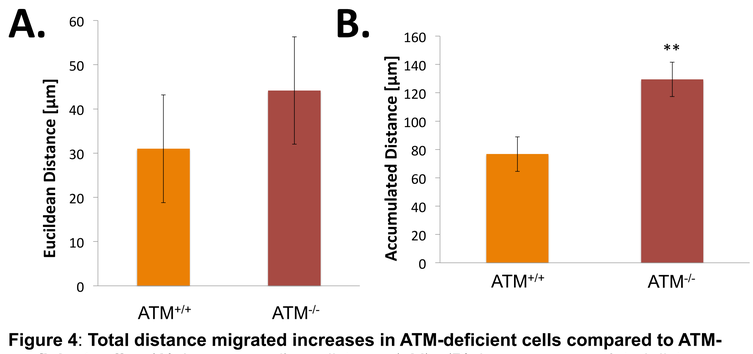
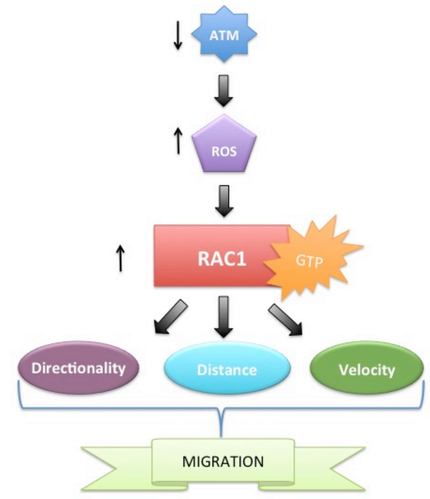


Figure 4: **Total distance migrated increases in ATM-deficient cells compared to ATM-proficient cells**. **(A)** Average eucilean distance (µM). **(B)** Average accumulated distance (µM). Fibroblasts were plated on fibronectin coated coverslips and imaged every 10 minutes over 16 h with a Nikon BioStation IM (20X Objective). Total accumulated distance was measured with NIH Image J software using the manual tracking plug-in. Error bars represent the S.E.M. and the student's *t*-test for paired samples was used to determine ** $p < 0.05$.

Working Hypothesis



Future Directions

- To analyze the contributions of GEFs, GAPs, and RhoGDIs in Rac1 activation in ATM proficient and deficient cells.
- To analyze and assess the effects of Rac1 activity on ATM proficient and deficient cells.
- To analyze the effects of Rac1 activity on cellular migration using siRNA knock-down in $ATM^{+/+}$ and $ATM^{-/-}$ cells.
- To evaluate the activity of Rac2 and Rac3 in ATM proficient and deficient cells.
- To evaluate the therapeutic applications of targeting Rac1 with the selective Rac inhibitor NSC23766

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References

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