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A new role for ATM

Regulating mitochondrial function and mitophagy

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The various pathologies in ataxia telangiectasia (A-T) patients including T-cell lymphomagenesis have been attributed to defects in the DNA damage response pathway because *ATM*, the gene mutated in this disease, is a key mediator of this process. Analysis of *Atm*-deficient thymocytes in mice reveals that the absence of this gene results in altered mitochondrial homeostasis, a phenomenon that appears to result from abnormal mitophagy engagement. Interestingly, allelic loss of the autophagic gene *Becn1* delays tumorigenesis in *Atm*-null mice presumably by reversing the mitochondrial abnormalities and not by improving the DNA damage response (DDR) pathway. Thus, ATM plays a critical role in modulating mitochondrial homeostasis perhaps by regulating mitophagy.

Ataxia telangiectasia patients display numerous pathologies that develop early in life, including cerebellar ataxia, insulin resistance, radiosensitivity, and T-cell lymphomagenesis. A-T is caused by deficiency in the *Ataxia Telangiectasia Mutated (ATM)* gene, which encodes a serine/threonine protein kinase that plays a role as a master mediator of the DNA damage response to double-strand breaks. Accordingly, the overall A-T pathology has mainly been attributed to defects in the DDR. However, the fact that some of the abnormalities seen in A-T patients are difficult to explain by defects in the DDR led us to hypothesize that ATM might have additional non-nuclear functions that contribute to the A-T disease. To investigate this possibility, we analyzed the thymus from *Atm*-null mice since this is the major site of tumorigenesis in A-T patients and *Atm*-deficient mice. Strikingly, we observed that loss of *Atm* in thymocytes results in numerous mitochondrial abnormalities, including altered morphology and deficiencies in mitochondrial electron transport chain activity that correlates with impaired ATP levels and elevated mitochondrial ROS.

Interestingly, *Atm* loss also leads to an elevated mitochondrial mass and membrane potential that correlates with an increase in oxygen consumption within the thymus. To elucidate the causes of the increase in mitochondrial mass in the absence of *Atm*, we further investigated the dynamic of this organelle. Our data suggested that the increase in mitochondrial mass in *Atm*-null cells does not result from changes in mitochondrial biogenesis, but rather from defects in the selective clearance of damaged mitochondria by autophagy (mitophagy). Interestingly, despite displaying impaired mitophagy, *Atm*-null cells are capable of triggering conventional autophagy as assessed by analysis of autophagic markers, and actually exhibit increased basal autophagy markers. Thus, there is a unique situation of increased autophagy and decreased mitophagy in *Atm*-null thymocytes.

Induction of mitophagy following mitochondrial damage is dependent on accumulation of PARK2/PARKIN at the mitochondria. In accord with this model, we observed that CCCP treatment of normal human fibroblasts results in increased levels of mitochondrial PARK2. Interestingly, *ATM*-null fibroblasts appear to possess high levels of endogenous PARK2 protein that localize to the mitochondria even in the absence of CCCP treatment.

Keywords: *Ataxia Telangiectasia Mutated* gene, mitochondrial dysfunction, PARKIN, BECN1, mitophagy

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Additionally, expression of exogenous PARK2 also tends to localize to the mitochondria of *ATM*-null fibroblasts under normal conditions, while normal fibroblasts display a more cytoplasmic distribution. These data further support a model in which cells lacking *ATM* display defective mitochondria, and that the process of mitophagy is impaired in these cells leading to an increase in mitochondrial number. It is intriguing that accumulation of PARK2 at the mitochondria of *ATM*-deficient cells is not sufficient to engage the process of mitophagy. It is possible that although PARK2 can translocate to the mitochondria upon mitochondrial damage, its function might be impaired in the absence of *ATM*.

The increased oxidative damage and altered autophagy/mitophagy observed in the absence of *ATM* prompted us to further investigate whether changes in the autophagic process might influence the phenotype observed in *Atm*-null mice. To this point, we bred the *Atm*-null mice into a *Becn1* heterozygous background. Surprisingly, loss of one *Becn1* allele in the *Atm*-null background results in a significant delay in the tumorigenic phenotype of these mutants. Further analyses indicated that the partial rescue of tumor phenotype in these compound animals was not due to an improvement in the DDR pathway, but rather to a rescue of the mitochondrial abnormalities seen in *Atm*-deficient mice. However, the impact of *Becn1* heterozygosity on cell fate appears to be context-dependent since *Becn1* heterozygosity increases the tumor onset in a mouse model of Burkitt lymphoma. Thus, our data suggest that *Becn1* cannot simply be considered as a tumor suppressor. Importantly, our data imply that the tumorigenic phenotype seen in the absence of *ATM* is not purely a consequence of an impaired DDR mechanism, but also is due to defects in mitochondrial homeostasis perhaps by the inability of these cells to engage mitophagy. Moreover, the rescue of the mitochondrial abnormalities in *Atm*-null cells by *Becn1* heterozygosity suggests that BECN1 might have additional roles rather than regulating the autophagic pathway.

It would be of great interest to examine if other components of the autophagic pathway, such as ATG5 and ATG7, also alter the tumorigenic and mitochondrial phenotypes in *Atm*-deficient mice. Further, studying the deficiencies of these genes in other tissues such as the central nervous system might elucidate if alterations in the autophagy and/or mitophagy processes contribute to the development of other A-T pathologies such as neurodegeneration. Additionally, since ATM is a serine/threonine kinase, it would be interesting to study whether ATM regulates mitochondrial homeostasis and perhaps mitophagy by phosphorylating key players in these processes. The fact that this study also revealed that a fraction of ATM localizes to mitochondria and that it becomes activated during mitochondrial damage supports this model.