# Atm functions to address dsbs

During the course of a cell’s lifetime, it will be exposed to numerous DNA-damaging events due to various chemical and environmental agents. Should a cell be rendered unable to repair the damage to its genetic material, cancer and other maladies may develop, which may ultimately lead to the death of the organism. (SUZANNE CLANCEY). To this extent, numerous human diseases are attributed to an inability to repair DNA damage. Among these diseases, ataxia telangiectasia is perhaps one of the most pertinent examples. It is an autosomal disease caused by inactivation the ataxia telangiectasia mutated (ATM) protein, where the disease serves as the protein’s namesake. At present, ATM is believed to be responsible for initiating the phosphorylation wave of the DNA damage response in response to double-strand breaks (DSBs), which are often particularly damaging to the cell’s genomic integrity due to the fact that they often result in unfaithful DNA repair. These DSBs are also each unique in their structure and character, where the ends of the location of the DSB may not follow the typical 5’ phosphate and 3’ hydroxyl DNA structure, and thus an array of mechanisms may be required to repair the break. However, certain cuts may not lend themselves well to being repaired, and thus may be “ blocked” from undergoing DSB repair and the only solution would thus be to sever off the blocked portion via nuclease action. But in order to study this phenomenon, it would require all DSBs to be similar in nature, which is not the case for most mutagenic agents.

To this end, Alvarez-Quilon and colleagues utilized a particular drug known as etoposide, which is an anticancer drug that is capable of acting as an inhibitor to an enzyme known as DNA topoisomerase II (TOP2) that is able to

relax and unknot DNA molecules. However, during its mechanism of action, it is required to pass duplex DNA through a temporary DSB created by the enzyme. Here, two subunits of the TOP2 are linked to each 5’ end of a DSB via a phosphodiester bond. If this intermediate becomes stabilized, then transcription can become interrupted and thus forces the TOP2 to be degraded and leaving behind permanent DSBs, each with peptide-based impediments at the 5’ ends of the DNA. Thus, etoposide is capable of providing a consistent type of DSB, all of which have 5’ end blockages. The only enzyme that is understood to remove this type of blockage is tyrosyl DNA phosphodiesterase 2 (TDP2), and thus should this enzyme be damaged via a mutation, then any blockage produced is irreversible and requires nucleases to allow repair processes to proceed. Thus, two types of DSBs can be created: one with a pure DSB without any blockage via applying etoposide to wild-type cells (which have TDP2 intact), and another with blocked DSBs via etoposide treatment of TDP2-mutant cells.

Thus, the question the authors sought out to was how and when ATM functions to address DSBs. They hypothesized that ATM functions to rejoin blocked DSBs strictly when the ends were irreversibly blocked by the effect of etoposide on TOP2.

In order to find support for their hypothesis, numerous experiments were performed by Alvarez-Quilon and colleagues. One experiment the researchers performed was to analyze what would happen to the repair of clean or blocked DSBs should ATM loss occur. To do this, Alvarez-Quilon and colleagues used mouse embryo fibroblasts (MEFs) that were exposed to an etoposide treatment, confluently halted at the G0/G1 cell growth stages, and

analyzed Ser139-phosphorylated H2AX (γH2AX) foci disappearance via immunofluorescence. Since ATM is responsible for phosphorylating H2AX, the disappearance of fluorescence indicates that ATM is not functioning or present. Overall, it was observed that there was a nearly negligible difference in repair rate between cells with or without TDP2 (shown in Figure 2a). Cells with present TDP2 and damaged ATM did not greatly affect DSB repair. However, when both TDP2 and ATM were damaged, a large drop in the repair rate was noted. The same results were observed when using either an ATM chemical inhibitor or when using MEF cells from patients with ataxia telangiectasia (Figure 2b and 2c). These results overall suggest that ATM is indeed important for the repair of DSBs with blocked ends, as shown by the significantly lower repair rate in the cells without either TDP2 and ATM, while this was not seen in cells where one or both functioned.

Next, the researchers wished to see if ATM function on blocked DSBs has consequences for the cell. To do this, they monitored cell survival rate in response to various concentrations of etoposide. What they found was that cell survival had greatly decreased for cells lacking both TDP2 and ATM, as made evident by the fact that they were most susceptible to a lower growth rate when exposed to increasing concentrations of etoposide (Figure 4a). This result was more amplified when an ATM inhibitor was used, with an even lower growth rate being obtained relative to cells with one or both of ATM or TDP2 present (Figure 4b). This may suggest that chemical inhibition of ATM is worse for a cell than a deletion of the protein as a whole, and that a non-functioning ATM may interfere with processes related to cell growth and repair.

In addition to the cell growth rate, the genome stability was also analyzed after etoposide treatment in MEFs with and without TDP2 protein which induced micronuclei formation (caused by mis-segregation of chromosomes or acentric chromosomal fragments were present and thus indicative of genome instability). In order to restrict the analysis performed and increase the consistency of the results, only binucleated cells that had blocked cytokinesis as a result of chemical treatment were scored (Figure 5a). Overall, the amount of chromosomal aberrations increased when either ATM or TDP2 were deleted (Figure 5b). However, when both ATM and TDP2 were deleted in the same cell, the amount of chromosomal aberrations became much greater in number than with just one of ATM or TDP2 missing. Overall, it can be said that these results serve to show ATM-mediated repair allows for an increased ability of cells to survive, as well as to stabilize the integrity of the genome as a result of blocked DSBs.

As a whole, it can be said that this paper provided an important understanding in the role of ATM in DSB break repair. The potential for this research mainly lies in its ability to improve understanding of ataxia telangiectasia, with the potential that new avenues of disease treatment may be discovered. In addition to this, this paper contributed to a greater understanding of DNA repair, which can provide insights into additional mechanisms of repair in other organisms.

Furthermore, this paper had generally utilized appropriate controls and experiments which improved the validity of the results obtained . However, one major issue with it is the fact that only one type of chemical was utilized in order to induce blocked DSBs. While it is convenient that it only had one

known particular function (to disable TOP2), it is a possibility that etoposide has other effects in the cell. Using another topoisomerase inhibitor may provide a broader picture as to whether or not ATM affects blocked DSBs. Furthermore, only one type of block was observed (that of TOP2). Could other types of blocks have different requirements, perhaps ones not needing ATM, or even being less reliant on it? Other types of chemicals may help create different blocks, which could then be analyzed for ATM function. Should these additional experiments be performed and again come back with the result that ATM functions to remove blocked DSBs, this would provide greater credence to the findings of this paper.

## References

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