ABSTRACT

Cancer is a complex of diseases caused by mutational alterations of normal cells and is a leading cause of death worldwide. Transforming events in the context of cancer include deregulated expression or activation of oncogenes and inactivation of tumor suppressors, one of which is *ATM*. The ATM kinase serves as a guardian of genome stability, playing a key role in the DNA damage response (DDR). Activated by DNA double strand breaks (DSBs), ATM triggers a number of signaling cascades, inducing cell cycle arrest and DNA repair via homologous recombination (HR) or, in cases of excessive damage, apoptosis. ATM-deficiency on the organismal level is characterized by a complex phenotype that involves reduced overall survival, enhanced radiosensitivity, and predisposition to cancer, mainly of hematopoietic origin. ATM-deficient cancer cells strongly rely on non-homologous end joining (NHEJ) and its central player, DNA-PKcs (DNA-dependent protein kinase, catalytic subunit), as a dominant remaining pathway to repair DSBs. Thus, DNA-PKcs is a promising target in ATM-deficient cancers. Though cancer-related *ATM* inactivation is typically associated with poor prognosis, it is currently unknown whether ATM-deficient cancer cells rely on a consistent absence of ATM. Examples from related tumor suppressors, such as *TP53* and *BRCA2*, show that restoration of the missing tumor suppressor can lead to dramatic tumor regression (*TP53*), but can also be tolerated by malignant cells (*BRCA2*).

In this work, we aim to characterize DNA-PKcs as a therapeutic target in ATM-defective cancers and to investigate the role of ATM re-expression in initially ATM-deficient malignancies.

In a set of experiments performed in ATM pro- and deficient settings, we were able to confirm the addiction of ATM-deficient human and murine cells to DNA-PKcs. We could also show the tolerance of ATM-wildtype cells to DNA-PKcs inhibitors, illustrating the advantages of these molecules as potential therapeutics, due to the lack of toxicity in normal tissues. We further revealed the inability of ATM-deficient cells to repair DNA lesions when DNA-PKcs was inhibited. Finally, we demonstrate that DNA-PKcs inhibitors are effective in an *Atm*-defective murine model of *Myc*-driven B-cell Non-Hodgkin Lymphoma (B-NHL), and can prolong survival of lymphoma-bearing mice.

In order to investigate the biological effects of ATM re-expression in cancer, we generated a reactivatable *Atm* allele, which phenocopies *Atm*-knockout mutants with regard to radiosensitivity, defects in immune cell maturation, and cancer predisposition. We show that *Atm* reactivation in autochthonous T cell lymphomas and *Myc*-driven B-NHLs induces lymphoma regression. We further employed transplantation experiments to distinguish between *Atm* reactivation in cancer cells and non-cancerous tumor stroma, and revealed both cell autonomous and non-cell autonomous effects of ATM re-expression.

Together, our data suggest that DNA-PKcs is a potential drug target for the treatment of ATM-defective malignancies. Furthermore, this data served as a background for initiation of a phase I clinical trial, aiming to assess the safety and tolerability of a dual DNA-PKcs and mammalian target of rapamycin (mTOR) inhibitor for patients with advanced solid tumors and hematologic malignancies. Our results also demonstrate that a continued absence of *Atm* expression is critical for the *in vivo* maintenance of an oncogenic state in B- and T cell lymphomas. In addition, our data indicates that impaired immune surveillance contributes to cancer predisposition in Ataxia-telangiectasia (A-T) patients, suggesting transplantation of ATM-proficient immune cells as a possible approach for cancer treatment in this patient group.