Exploiting DNA damage repair defects for effective targeting of acute myeloid leukaemia by PARP inhibitors

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Introduction

Inhibitors of Poly-ADP-Ribose Polymerase (PARPi) have been successfully developed and approved by FDA for treatment of ovarian cancer carrying mutations in DNA damage response (DDR) genes *BRCA1/BRCA2*[1-3]. In Acute Myeloid Leukemia (AML) patients, these mutations are extremely rare. However, chromosomal rearrangements generate chimeric oncofusion proteins [4] that, by acting as transcriptional regulators, impair DDR gene expression[5, 6]. This prompted us to test the efficacy of using PARPi in AML.

Materials and Methods

PARPi were tested *in vitro* and *in vivo*. *In vitro* experiments were carried out in mouse and human leukemic cell lines. Mouse leukemic cells expressing the oncofusion gene of interest were generated by Retroviral Transduction Transformation Assay (RTTA)[7].

Results

Leukemic cells driven by the oncofusion genes, AML1-ETO and PML-RARα, are sensitive to PARP inhibition whereas cells harbouring MLL-AF9 translocation are resistant[8]. Treatment of AML1-ETO and PML-RARα leukemic cells with PARPi induces apoptosis, senescence, cell cycle arrest and differentiation *in vitro* and significantly prolongs survival *in vivo*. By using γH2AX and RAD51 as markers of DNA damage and HR (Homologous Recombination)

we showed that AML1-ETO and PML-RARα cells accumulate DNA damage and are defective in recruiting RAD51 to DNA damage foci upon PARPi treatment. Further analysis revealed that the expression of a number of genes that are involved in the HR pathway are reduced in AML1-ETO and PML-RARα cells including *Rad51*, *Brca2* and *Rpa1*. This suggests that AML-ETO and PML-RARα are sensitive to PARPi as result of defective DDR. We showed that HOXA9, a key downstream target of MLL-fusions plays a critical role in promoting expression of HR genes and thus providing evidence by which MLL-AF9 are resistant to PARPi[9]. Depletion of Hoxa9 reduces the expression of *Rad51* and *Brca2* in MLL-AF9 cells and confers PARPi sensitivity in MLL-AF9 leukemic cells, compromising its ability to form colonies, repair DNA damage and prolongs survival in mouse models. Conversely, HOXA9 overexpression rendered AML1–ETO and PML–RARα cells resistant to PARPi. Likewise, pharmacological suppression of *Hoxa9*, using GSK3 inhibitor LiCl, can also sensitize MLL-AF9 cells to PARPi and prolongs survival in our mouse model.

Discussion

Our data indicate that PARPi might offer a new therapeutic strategy for patients with AML1-ETO or PML-RARα translocations. More importantly, we showed for the first time that HoxA9 can activate a potential DNA repair back-up pathway. PARPi in combination with pharmacological inhibitors of HOXA9 may represent a novel avenue for tailored therapeutic targeting of the aggressive MLL leukaemia[8].

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