AsiDNA™ induces tumor sensitivity to PARP inhibitors in homologous recombination proficient breast cancer

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Abstract

Objectives: PARP inhibitors (PARPi) have shown significant benefits in patients with BRCA-mutated tumors. However, their major limitations are the necessity of homologous recombination (HR) deficiency and the rapid emergence of resistance. In the current study, we explored a novel therapeutic strategy, based on drug combination to promote sensitivity to PARPi independently of the tumor genetics.

Experimental Design: We used AsiDNA, a DNA repair inhibitor (Dbait concept), consisting in small molecules mimicking double-strand DNA breaks to activate extensive glycolysis of DNA damage and prevent recruitment at damage sites of HR repair enzymes. We characterized the inhibition activity of AsiDNA on monitoring repair foci formation and DNA damage and analysed the cell survival to AsiDNA monotherapy and combination with PARPi Olaparib of 22 tumor cell lines, and 5 non-tumor cell lines. In vivo efficacy of the combined treatment was analyzed in tumor xenografts derived from the NDA-MB-231 cell line showing reduced sensitivity to AsiDNA and Olaparib in vitro. Frequency of clones resistant to two weeks treatment with AsiDNA or Olaparib was quantified in the 4NQO-mutagenized Hepa1C1c7 cells.

Results: Molecular analyses of repair foci formed after irradiation demonstrate that AsiDNA elicits recruitment of RAD51 and 53BP1 whereas Olaparib prevents HRR. Combination of both drugs increases the accumulation of unrequited spontaneous damage leading to an increase of all cell line subsets. The synergy of the association of AsiDNA with PARPi was also confirmed with 6 other PARPi. AsiDNA does not induce any increase in DNA damage or lethality in non-tumor cells when used in monotherapy as well as in association with PARPi. Selection of resistant clones allowed the emergence of resistance to Olaparib (1.4%), Imatinib (0.6%) and 6-TG (1.4%) but not to AsiDNA (0.07%), indicating that the drug is able to block several DNA repair pathways. In the NDA-MB-231 xenograft model, Olaparib alone failed to prevent tumor growth while AsiDNA provided 50% increase in tumor growth delay. Interestingly, the combination of Olaparib and AsiDNA increased 230% the tumor growth delay.

Conclusion: Our results highlight the therapeutic interest of combining AsiDNA and PARPi to recapitulate synthetic lethality in all tumor subtypes independently of HR status. Moreover, the low frequency of appearance of resistant clones to AsiDNA suggests a sustained efficacy during treatment unlike most targeted therapies.

Molecular mechanisms underlying the combination of AsiDNA and PARPi

Inhibition of Rad51 recruitment at damage site

Olaparib (Ola) and AsiDNA are both DNA repair inhibitors inhibiting the recruitment of repair enzymes at damage sites.

Accumulation of damage in malignant cells but not in normal cells

AsiDNA inhibits the repair of Ola induced DNA damage

AsiDNA acts specifically on tumor cells

Introduction

Multiple DNA Repair pathways inhibition by AsiDNA

Olaparib is short and stable DNA molecules that mimic DSBs. AsiDNA, a molecule of Dbait family, acts by hijacking and hyper-activating the DNA-dependent protein kinases (DNA-PKcs), leading to the hyperphosphorylation of DNA-PKcs and the increased recruitment of DNA-PKcs to DSBs (PARPi), which modify the chromatin and consequently the inhibition recruit the mitochondrial at the damage sites of many proteins involved in the CSR (HRR and NHEJ) and SSB (NER) repair pathways. This strategy sensitizes tumors to DNA damaging therapies such as radiotherapy and chemotherapy.

Repair inhibition by PARPi inhibitors (PARPi)

In cells, damage which arise during normal cellular activity, are repaired by the base excision repair pathway (BER) involving PARPi. PARPi inhibition leads to the accumulation of unrepaired single-strand breaks (SSBs) that result in stalled replication forks, collapsed chromosomes, genomic instability, and/or cell death. These SSBs are mainly repaired by Homologous Recombination (HR). Cells with BRCA2/2 mutations are defective in HR (so-called BRCA-deficient) and are sensitive to PARPi. Cells possessing HR, accurately and efficiently repair DSBs, and are less sensitive to PARPi.

Rational for combining AsiDNA and PARPi

AsiDNA induces a transient «Homologous Recombination Deficient» status that would render all cells sensitive to PARPi.

Multiple DNA repair pathways and PARPi inhibitors

Schematic representation of the combined treatment of AsiDNA and Olaparib.

AsiDNA stimulates PARPi efficacy independently of the genetic of the tumors

AsiDNA+Olaparib: combined treatment show better tumor growth delay and longer survival compared to single treatment

Chemical mutagenesis and selection of resistant clones

Low occurrence of clones resistant to AsiDNA

Molecular effects are observed with PARPi, targeting catalytic activity or trapping PARPi at damage sites

Synergy of the association of AsiDNA and Olaparib in a HR proficient Breast Cancer model

AsiDNA+Olaparib: combined treatment show better tumor growth delay and longer survival compared to single treatment

AsiDNA alone: no effect on tumor growth

AsiDNA is synergic with all PARPi inhibitors

Low occurrence of clones resistant to AsiDNA

> Synergistic effect between AsiDNA and Olaparib in triple negative breast cancer cell lines.

> No toxicity of the combined treatment in normal mammary cell lines.

Resistant clones appear during 6-TG, Imatinib and Olaparib but not with AsiDNA treatment.