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VRK1 depletion is associated to a defective DNA damage response after combining ionizing radiation and PARP inhibitors.

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Introduction: Eukaryotic cells are continuously exposed to exogenous and endogenous agents, which are responsible for inducing DNA damage. In order to prevent the adverse consequences of these DNA lesions in the integrity and stability of genetic information, higher organisms have developed DNA damage signaling and repair machineries adapted specifically to the type of damage.
Defects in DNA repair, along with the effect of the DNA damage itself and the failure to stall or stop the cell cycle before damaged DNA is passed on to daughter cells, can lead to genomic instability, a typical feature of cancer. Because of that, inhibitors of DNA damage response (DDR) pathways and agents that induce DNA breaks have become an effective anticancer therapy.
PARP-1 (Poly (ADP-ribose) polymerase 1) is an important protein involved in DNA repair and transcriptional regulation and is recognized as a key regulator of cell survival and cell death. In turn, it is considered a master component of a number of transcription factors implicated in tumor development and inflammation. As a DNA-binding and chromatin-associating enzyme, it mediates single-strand DNA break repair, alternative end-joining of DNA double-strand breaks, and also aspects of homologous recombination (HR). For this reason, pharmacologic inhibition of PARP-1/2 is synthetically lethal in association with genetic or functional defects in BRCA1/2 and other genes involved in HR. Furthermore, this inhibition sensitizes malignant cells and tumors to ionizing radiation (IR), although it is poorly understood the mechanisms through which PARP inhibition causes radiosensitization.
Recently, VRK1 (the most abundant kinase in chromatin) has been described to be required for the assembly of 53BP1 foci and participates in the recruitment and formation of γH2AX foci in response to ionizing radiation. This kinase also phosphorylates and protects Nbs1 to proteosomal degradation after inducing DNA damage, and, moreover, it interacts with p53 and is activated by UV-induced DNA lesions. Therefore, it is possible that VRK1 plays a critical role in several steps of DDR after combining IR and PARP inhibitors.

Objectives: Our first aim is to determine how Olaparib (PARP inhibitor widely employed in cancer treatment) and IR dosages might be reduced after their combination in order to decrease the side effects on patients, and, secondly, whether VRK1 depletion interferes with DDR in response to DNA damage induced by these two anticancer treatments.

Methods: To study the effect of combining Olaparib and IR on foci formation, cells were treated with increasing dosages of this PARP inhibitor and irradiated using rising doses afterwards. Then, these cells were fixed and the assembly of DNA repair foci was assessed by immunofluorescence. On the other hand, VRK1 knockdown was performed using different siRNAs and VRK1 levels were tested by immunofluorescence and western blot.

Results: Based on the assembly of γH2AX and 53BP1 foci, we can conclude that 5 μM Olaparib and IR 1 Gy is the most effective combination of both anticancer treatments, since the number of these foci is comparable to higher dosages of Olaparib or IR separately in three different tumor cell lines. Later, VRK1-depletion experiments show a drastic reduction of DNA repair foci (γH2AX, Nbs1 and 53BP1) after inducing DNA damage by Olaparib, IR or their combination, both in presence or in absence of serum. Furthermore, the consequences associated to VRK1 knockdown are reproducible in p53- and ATM-null cells. Finally, H4K16

acetylation levels, which are directly related to local relaxation of chromatin in response to DNA lesions, are also decreased significatively in absence of this kinase.

Conclusions: Overall, these results indicate, first of all, that the combination of PARP inhibitors like Olaparib and IR allows the reduction of dosages for each treatment, with a positive impact on cancer patients, and, in second place, VRK1 depletion is correlated with a defective DNA damage response after inducing DNA damage with IR and/or Olaparib, which is independent of p53 or ATM, essential in other DNA repair pathways. As a consequence of this, VRK1 chromatin kinase could become a new therapeutic target in current approaches against cancer.

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