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VRK1 is required for the DNA damage response induced by temozolomide and by intercalating agents blocking transcription.

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Introduction: Chromatin undergoes dynamic changes in both normal cellular processes (cellular division, transcription and replication) and in pathological situations (DNA damage, among others). DNA is constantly exposed to exogenous (environmental and chemical factors) and endogenous damage agents (oxidative stress) which cause lesions. In order to repair those lesions, cells have developed several mechanisms that take part in a global process called DNA damage response (DDR), essential to maintain genome integrity and homeostasis. Unrepaired lesions could lead to cancer and neurodegenerative diseases.
Vaccinia related kinase 1 (VRK1) is a nucleosomal serine threonine kinase that has been proved to play an important role in DDR by participating in the assembly of 53BP1 and γH2AX foci and by forming a stable complex with NBS1 after gamma radiation exposure. Moreover, VRK1 participates in the epigenetic changes that are necessary for chromatin remodeling after DNA damage.

In this study, we have used two drugs that induce different types of DNA damage: temozolomide and actinomycin D. Temozolomide is an alkylating agent that causes DNA methylation and is in use for glioblastoma treatments. Actinomycin D intercalates into the DNA causing transcription inhibition. In addition, we have used olaparib, a PARP inhibitor, in order to study whether it sensitizes temozolomide effect on glioblastoma cells.

Objectives: Our aim is to determine the role of VRK1 in the DNA damage response when the DNA is exposed to two drugs that cause different types of damage: DNA methylation by alkylating agents and transcription inhibition by actinomycin D.

Methods: To study VRK1 response, cells were treated with temozolomide 200 μM and actinomycin D 70 nM (separately), and 53BP1, NBS1 and the acetylation of histone H4 were studied using immunofluorescence techniques. VRK1 was depleted to study its effect on these damage markers after DNA damage. Moreover, temozolomide doses was reduced (50 μM) when combinated with olaparib 5 μM.

Results: In our study, we have observed that the acetylation of the histone H4 increases after 5 minutes of both types of damage (transcription inhibition and DNA methylation), which means that the chromatin is reorganized in order to be repaired. In addition, we have noticed that NBS1 increases after 15 minutes and the number of 53BP1 foci also increases after 30 minutes of both treatments. NBS1 is an early component of the DDR pathway and 53BP1 is part of a specific repairing route. Therefore, these results suggest that VRK1 participates in several DDR steps. Besides, VRK1 depletion reduces the acetylation of histone H4, necessary for chromatin remodeling before DNA damage response, and it affects 53BP1 foci formation, a marker of DNA double-strand breaks. Depletion of VRK1 impairs the DDR to treatment with olaparib (PARP inhibitor) and/or temozolomide, and thus compromise cell viability.

Additionally, we have observed that the combination of temozolomide and olaparib allow us to reduce both doses getting the same effect, which can result in a reduction of their toxicity.

Conclusion: Based on these results, we conclude that VRK1 participates in the DNA repair process after actinomycin D and temozolomide treatments. Furthermore, VRK1 could participate in the early damage response because the acetylation of the histone H4, necessary for chromatin remodeling before repairing, is reduced by VRK1 depletion. Moreover, we suggest

that temozolomide, in combination with olaparib, could have a stronger effect at lower doses on glioblastoma cells indicating that the toxicity associated with these drugs can be reduced.

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