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VRK1-mediated phosphorylation promotes Tip60/KAT5 accumulation and activity in response to DNA damage

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Introduction: Eukaryotic DNA is highly organized in a packaged nucleoprotein structure known as chromatin. The genomic information included in this chromatin must be maintained unalterable in order to confer a stable cell identity on tissues and prevent diseases development, including cancer. For that, cells have developed a complex DNA damage response (DDR) that ensures genome integrity in response to different types of DNA lesions. In this respect, epigenetics plays a crucial role at different levels of the DDR: first, the addition of different chemical modifications to specific histone residues enables chromatin decompaction and guarantees the access of DNA-repair proteins to the lesion. Furthermore, other epigenetic modifications are essential for the proper progression of the DDR according to the type of DNA damage and the phase of the cell cycle in which that damage occurred. All these modifications (including acetylations, methylations, phosphorylations, ubiquitylations and others) are modulated by histone-modifying enzymes that belong to different families depending on the type of modification that they catalyze: histone acetyltransferases (HATs), deacetylases (HDACs), methyltransferases, demethylases, kinases, ubiquitin ligases, etc. However, how all these enzymes are coordinated in order to guarantee a proper DDR is not well-known.

Objective: Our main aim is to study the mechanism responsible for regulating histone-modifying enzymes during this process. In this context, we hypothesize that the chromatin kinase VRK1 might be involved in the regulation of these histone-modifiers, based on current data that show that the depletion of this Ser-Thr kinase prevents the addition of different epigenetic modifications (such as the acetylation on the lysine 16 of histone H4) required for the initial decompaction of chromatin during the DDR. Given this, we have focused our objectives on analyzing specifically how VRK1 is regulating the activity of the histone acetyltransferase Tip60/KAT5, since it is the enzyme responsible for catalyzing this acetylation of H4K16.

Methods: In order to study the role of VRK1 in the regulation of Tip60/KAT5 activity during DDR, cell cultures were treated with the chemotherapy drug doxorubicin 10 µM. After treatment, we use different experimental approaches, including Western-blotting and immunofluorescence assays, to analyze different Tip60 properties such as interaction with VRK1, phosphorylation state or stability. Furthermore, parallel experiments depleting VRK1 were also carried out to probe if this Tip60 properties change in absence of the kinase.

Results: Throughout our study, we have observed that VRK1 and Tip60/KAT5 form a basal complex in which the kinase rapidly phosphorylates the acetyltransferase only when DNA damage is induced with doxorubicin. This phosphorylation seems to be important for Tip60 accumulation during the DDR, since VRK1 depletion reduces Tip60 stability and impairs its chromatin accumulation in response to DNA damage. Finally, we studied the mechanism by which VRK1-mediated phosphorylation of Tip60 prevents its degradation. In this context, we have shown that levels of ubiquitylated Tip60 are reduced during DDR, which supports the fact that Tip60 accumulates in response to DNA damage. However, this decrease of ubiquitylated Tip60 after doxorubicin treatment is not allowed when VRK1 is silenced, indicating that VRK1-mediated phosphorylation could block Tip60 ubiquitylation and subsequent degradation via proteasome pathway.

Conclusion: Altogether, these results indicate that VRK1 may be a key component regulating Tip60/KAT5 activity, which could be essential for the development of a proper DNA damage response. From a clinical point of view, this approach can provide new insights on how to deal

with chemotherapy resistance by developing VRK1 inhibitors that increase the DNA damage accumulation in cancer cells and improve the efficiency of chemotherapy drugs.

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