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Original article

Knockdown of the nucleoporin Nup50 protects cells against ionizing radiation through enhancing DNA-PKcs-mediated DNA damage repair

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A B S T R A C T

Objective: To investigate the effect and mechanism of Nup50 on radiation-induced DNA damage repair to radiation and explore the potential role of Nup50 as radioprotective target.

Methods: The Nup50 gene was knocked down in HUVEC cells using lentiviruses. Colony formation, CCK-8, and flow cytometry were performed to determine the viability, proliferation and apoptosis of HUVEC cells treated with γ-rays , respectively. The extent of DNA damage was evaluated by using comet assay and immunofluorescence staining against γ-H2AX. In addition, we explored the role of Nup50 in DNA damage response (DDR) pathways through western blotting assay. Finally, nuclear and chromatin fractionation were performed to determine the potential molecular mechanism underlying the radiation protection function of Nup50 knockdown.

Results: Nup50 knockdown increased the cellular resistance to ionizing radiation. The CCK-8 data showed that cell viability was significantly increased in the Nup50 knockdown group after radiation ($t = 4.23$, $P < 0.01$). The Nup50 knockdown group also showed more survived colonies ($t = 10.06$, $P < 0.001$), less apoptosis rate ($t = 3.78$, $P < 0.05$) and less unrepaired DNA damage. Furthermore, Nup50 knockdown increased radiation-activated phosphorylation levels of DNA-PKcs in HUVEC cells. Finally, the nuclear and chromatin fractionation data showed that inhibiting Nup50 increased the recruitment of DNA-PKcs to chromatin after DNA damage.

Conclusions: Our findings revealed that Nup50 knockdown promoted radioresistance in normal HUVEC cells by regulating DNA-PKcs pathway, suggesting Nup50 as a potential target for radiation protection.

1. Introduction

As the wide application of nuclear techniques, radiation protection is drawing more and more attentions to ensure the safety of radiological works, as well as medical rescue for nuclear accident.[1] It has been reported that natural products, free radical scavengers, agonist of Toll like receptors, showed radioprotective property. However, no more radioprotective drug was approved till now other than amifostine (WR2721) in 1999.[2] In 2005, GM-CSF and G-CSF was approved by FDA in US, and listed in the WHO drug updates 2003.[3] It is required to identify novel mechanisms and targets of radiation injury repair to develop novel radioprotective techniques.

One of the main targets of ionizing radiation (IR) is DNA, and IR induces different types of DNA damages. Among these types, double-strand breaks (DSBs) are the most dangerous type of DNA lesions and pose a serious threat to genome instability because they can lead to the loss of large chromosomal regions.[4,5] The current model suggests that two major pathways, non-homologous end joining (NHEJ) and homologous recombination (HR), are responsible for repairing the majority of DSBs.[6–8] Compared to NHEJ, HR is essential for error-free DNA repair. One of the key initial steps in HR is the end resection of damaged DNA, which generates short, 3′-single-stranded DNA (ssDNA) which provides a platform for recruiting HR repair-related proteins and prevents DNA repair by NHEJ.[9,10] However, the mechanism on the regulation of radiation-induced DNA damage repair remains unclear.

The nuclear basket of nuclear pore complexes (NPCs) is composed of three nucleoporins: Nup153, Nup50 and Tpr. Nucleoporin 50 (Nup50) is an evolutionarily conserved protein that previously believed to be a structural component of the nuclear pore complex. Nup153 promotes nuclear import of 53BP1 in participated in DNA double-strand break repair. Nup50 contributes to proper DSB repair in a different manner from Nup153. Nup153 appears implicated in NHEJ and homologous recombination. Contrary to Nup153, loss of Nup50 only affected NHEJ.[11] Nup50 localizes to the nuclear side of NPCs and interacts with a number of nuclear transport factors, including importin α, importin β, transportin and RanBP7, RanGTP and probably CRM1.[12,13] It is a tristable switch that

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