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Promyelocytic Leukemia Proteins Regulate Fanconi Anemia Gene Expression

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Abstract: Promyelocytic leukemia (PML) protein is the core component of subnuclear structures called PML nuclear bodies that are known to play important roles in cell survival, DNA damage responses, and DNA repair. Fanconi anemia (FA) proteins are required for repairing interstrand DNA crosslinks (ICLs). Here we report a novel role of PML proteins, regulating the ICL repair pathway. We found that depletion of the PML protein led to the significant reduction of damage-induced *FANCD2* mono-ubiquitination and *FANCD2* foci formation. Consistently, the cells treated with siRNA against PML showed enhanced sensitivity to a crosslinking agent, mitomycin C. Further studies showed that depletion of PML reduced the protein expression of *FANCA*, *FANCG*, and *FANCD2* via reduced transcriptional activity. Interestingly, we observed that damage-induced CHK1 phosphorylation was severely impaired in cells with depleted PML, and we demonstrated that CHK1 regulates *FANCA*, *FANCG*, and *FANCD2* transcription. Finally, we showed that inhibition of CHK1 phosphorylation further sensitized cancer cells to mitomycin C. Taken together, these findings suggest that the PML is critical for damage-induced CHK1 phosphorylation, which is important for FA gene expression and for repairing ICLs.

Keywords: PML nuclear body; Fanconi anemia; interstrand DNA crosslink; CHK1 inhibitors

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1. Introduction

The promyelocytic leukemia (*PML*) gene was first discovered as a fusion partner of retinoic acid receptor α (RARα) in acute promyelocytic leukemia [1]. *PML* protein is the core component of multifaceted subnuclear structures known as *PML* nuclear bodies (*PML* NBs) that are implicated in the regulation of cellular functions including cell proliferation, apoptosis, senescence, tumor suppression, DNA repair, and DNA damage responses [2–6]. *PML* NBs of subnuclear spherical structure ranging from 0.1 to 1 μm in diameter contain diverse annotated domains, allowing them to interact with a variety of binding partners and facilitates their functions [2,7,8]. Based on more than a decade of studies, *PML* NBs are functionally associated with over 160 proteins directly and indirectly [3,9]. Seven *PML* isoforms, I~VIIb, have been characterized by their C-terminal ends, which determine their specific functions [10]. It was reported that the C-terminal of *PML* IV interacts with p53, which leads to the recruitment of p53 to *PML* NBs [11]. *PML* is also important for

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