CDK9 55k, A NEW PLAYER IN DNA DAMAGE REPAIR

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Introduction

DNA damage response (DDR) pathway plays a pivotal role in genomic stability. To better understand this network, our group performed a study to identified proteins involved in DDR. We identified the Cyclin Dependent Kinase 9 (CDK9) as a putative interactor of BRCA1, BARD1 and PTIP. CDK9 has two isoforms, the 42k form participates in transcription elongation and homologous recombination (through interaction with BRCA1 and BARD1). However, CDK9 55k isoform role remains unclear.

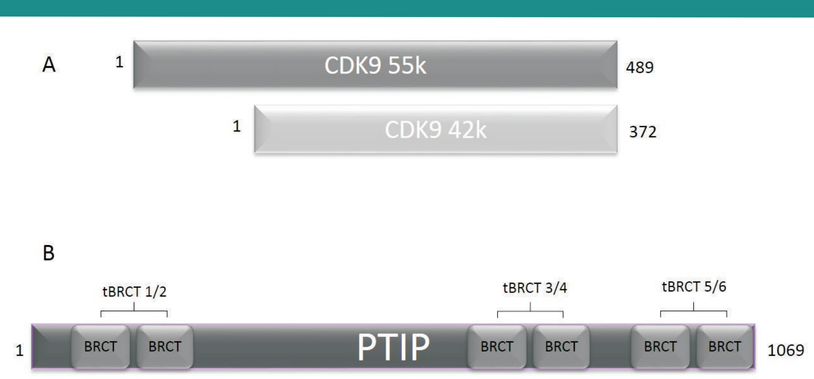

Figure 1. CDK9 isoforms and PTIP structure. (A) CDK9 55k and 42k, 117 amino acids in the N-terminal region differ CDK9 55k isoform from the 42k **(B)** PTIP encloses three tBRCT domains, which may play role in the CDK9/PTIP interaction.

Objective

In this work we intend to understand functional differences between CDK9 55k and 42k, exploring 55k interaction with PTIP and its possible role in non-homologous end joining (NHEJ) pathway.

Methods and Results

To evaluate CDK9/PTIP and CDK9/BRCA1 interactions, we performed GST-pulldown assays using PTIP tBRCTs or BRCA1 tBRCT and CDK9 55k or 42k ectopic expression in human cells. PTIP is involved in NHEJ, which occurs rather in G1 than S/G2 phases, therefore, we hypothesize that CDK9 expression may be regulated throughout cell cycle. To evaluate CDK9 55k and 42k levels over cell cycle, we synchronized BJ cells, harvesting cells in different phases to evaluate CDK9 by immunoblotting and real-time PCR. Pulldown assay demonstrate that both CDK9 isoforms interact with all three PTIP tBRCT domains, however only CDK9 42k interacts with BRCA1 tBRCT domain. Cell cycle analysis showed an extensive fluctuation of CDK9 55k protein levels throughout the cell cycle, displaying high levels of CDK9 55k in G1/G2/M in contrast with low levels observed in S phase. Interestingly, CDK9 42k and 55k mRNA levels showed no alterations throughout cell cycle.

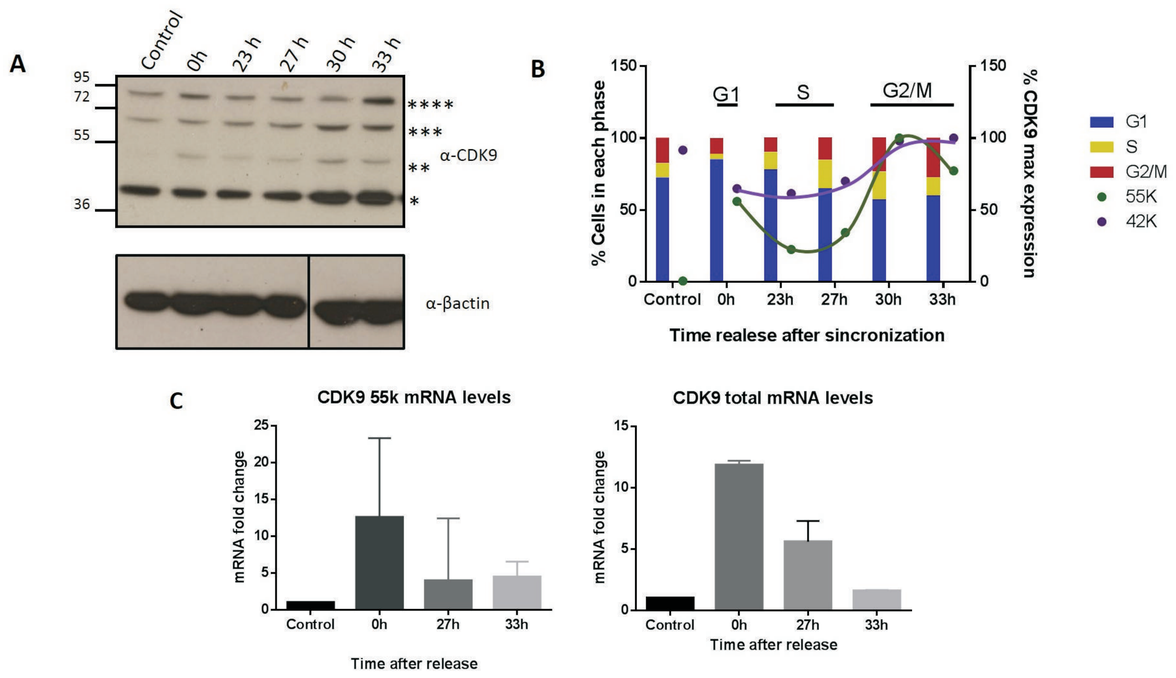

Figure 2. CDK9 expression profile throughout the cell cycle. BJ cells were synchronized in G1 and harvested at indicated time points after release. (A) CDK9 isoforms levels were evaluated by WB (upper panel). β-actin was used as loading control (lower panel). (*) CDK9 42k; (**) CDK9 55k; (***) and (****) are possibly CDK9 after post-translational modification. **(B)** Quantification of cells in each phase. Left axis indicates PI staining and flow cytometry analysis, which show the distribution in G1 (blue), S (yellow) and G2/M (red). Right axis indicates CDK9 levels, which were obtained by densitometry analysis of WB. Protein levels are depicted as green (55k) and purple (42k) dots. **(C)** Total mRNA was extracted using TRIZOL®. To verify 55k and total CDK9 mRNA levels, cDNA was obtained using High Capacity (and Random primers) and SuperScript II (and Oligo dT primers), respectively. CDK9 55k and total CDK9 mRNA were calculated by the ΔΔCT equation using the geometric mean of β-actin and GAPDH expression or β-actin alone as internal controls, respectively. Bars indicate mean +/- SD.

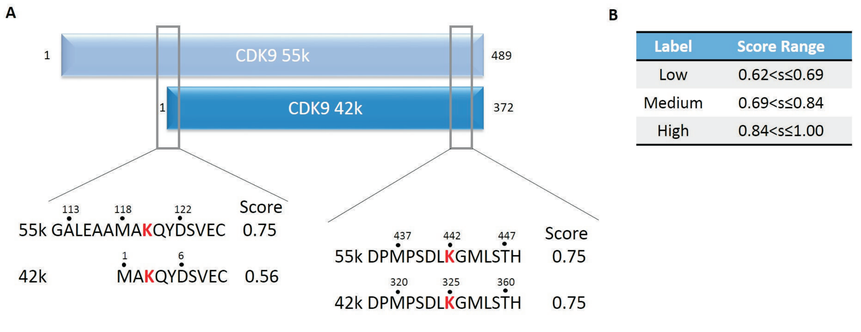

Figure 3. In silico analysis of putative ubiquitinated motifs. (A) CDK9 55k and 42k sequences were submitted to the UbiPred algorithm, to predict possible ubiquitylation sites. This analysis revealed two ubiquitylation motifs, one present in both proteins at the C-terminal region (0.75, medium confidence) and another restricted to the 55k isoform (0.75, medium confidence). **(B)** Labeled score for ubiquitylation prediction. Only scores above 0.62 are considered putative ubiquitylation sites

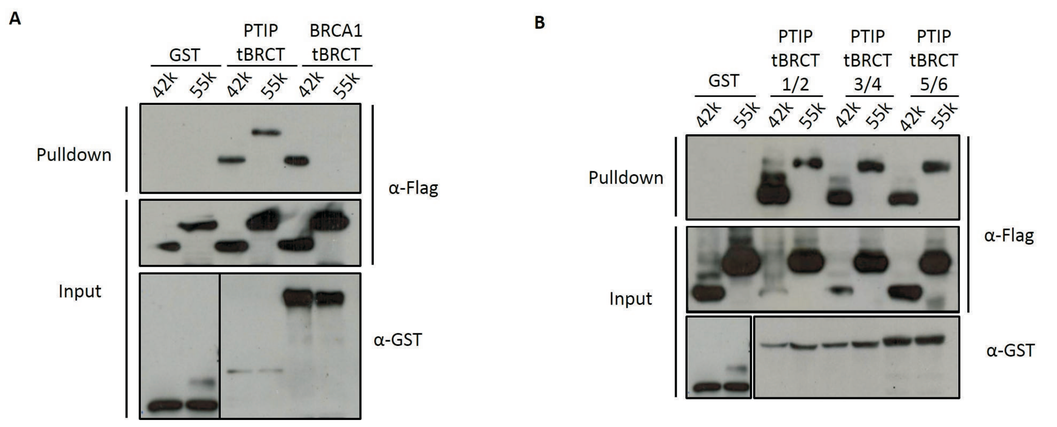

Figure 4. Both CDK9 isoforms interact with PTIP tBRCT. HEK293FT cells were co-transfected with Flag-tagged CDK9 (42k or 55k) and the indicated GST constructs. Cells were harvested 24 hours after transfection and extracts were used in pulldown assay. (**A; Upper panel**) Both CDK9 42k and 55k interacts with PTIP tBRCT (5/6), but only CDK9 42k interacts with BRCA1 tBRCT. Inputs are shown in lower panels. (**B; Upper panel**) Both CDK9 42k and 55k interacts with all PTIP tBRCTs. Inputs are shown in lower panels

Conclusions

Our data suggest that CDK9 55k is under a post-translational modulation along the cell cycle. Then, we intend to check CDK9 55k levels during cell cycle in the presence of cycloheximide and MG132 (a translation and a proteasome inhibitor, respectively). We are also generating a human cell line silenced for the 55k isoform using CRISPR/Cas9 technology to better access the impact of CDK9 55k absence in cellular processes.

Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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