Functional characterization CDK9 55k in DNA damage response

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INTRODUCTION

Genomic instability allows mutations in key genes that lead to carcinogenesis. DNA damage response (DDR) pathway plays a pivotal role restraining this event; thus, dysfunctional proteins involved in this pathway increase the risk of tumors occurrence. One domain commonly found in DDR proteins is the tBRCT. To study interactions in DDR, our group identified the tBRCT interaction profile of 7 different DDR-related proteins (BRCA1, BARD1, PTIP, ECT2, Ligase IV, MDC1, 53BP1). The cyclin dependent kinase 9 (CDK9) was identified as a putative BRCA1, BARD1 and PTIP tBRCT interactor. CDK9 was already reported to interact with cyclins T1, T2a, T2b and K, forming a complex called p-TEFb. Different from other CDKs, which play roles in regulating cell cycle transitions, the main function of p-TEFb complex is transcription elongation control. CDK9 has two isoforms: 55k and 42k. CDK9 42k interacts with ATR, in response to replication stress. Recently, our group reported CDK9 42k role in homologous recombination (HR) repair, critical for BRCA1 recruitment to DNA damaged sites. Curiously, it has already been reported the interaction between CDK9 55k and Ku70, that participates in non-homologous end joining (NHEJ) repair. PTIP, one of CDK9 tBRCT putative interactors also participates in NHEJ pathway.

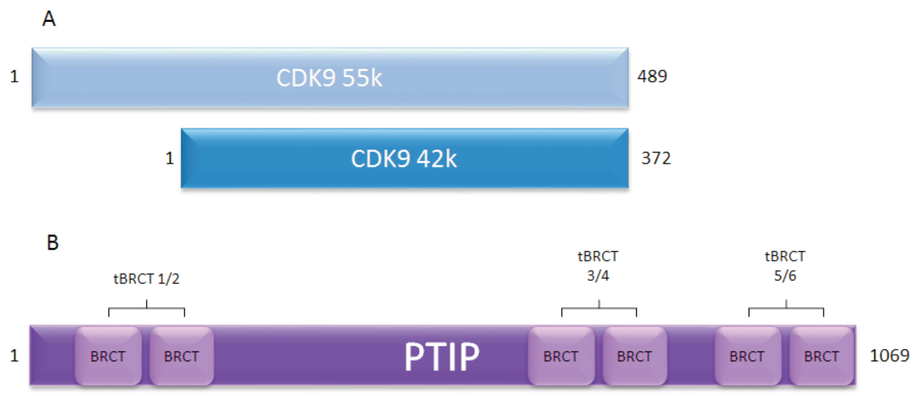

Figure 1: CDK9 isoforms and PTIP structure. (**A**) CDK9 55k and 42k, 117 amino acids in the N-terminal region differ CDK9 55k isoform from the 42k (**B**) PTIP encloses three tBRCT domains. Last tandem (C-terminal) was used in tBRCT interactome and possibly interacts with CDK9.

OBJECTIVE

In this study, we aim to understand functional differences between CDK9 55k and 42k, exploring 55k interaction with PTIP and its role in NHEJ.

METHODS

To confirm PTIP/CDK9 interaction, HEK293FT cells were co-transfected with CDK9 55k or CDK9 42k coding constructs, both Flag-tagged, and a construct enclosing one of PTIP tBRCTs (BRCT 1 and 2, BRCT 3 and 4 or BRCT 5 and 6) or BRCA1 tBRCT, all tagged with GST. Whole cell extracts were used in GST-pulldown assays and evaluated by immunoblotting. NHEJ pathway occurs rather in G1, while HR is observed in S/G2 phases. Therefore, we hypothesize that CDK9 expression may be regulated throughout cell cycle. To investigate CDK9 profile along cell cycle, we synchronized a human cell line (BJ cells), harvesting cells in different phases and evaluated CDK9 by immunoblotting.

RESULTS

Both CDK9 isoforms interact with all three PTIP tBRCT, however only CDK9 42k interacts with BRCA1 tBRCT. Co-immunoprecipitation assays are being performed to evaluate constitutive interactions.

Cell cycle data showed an extensive fluctuation of CDK9 55k levels throughout the cell cycle, showing high levels of CDK9 55k in G1/G2/M in contrast to low levels observed in S phase.

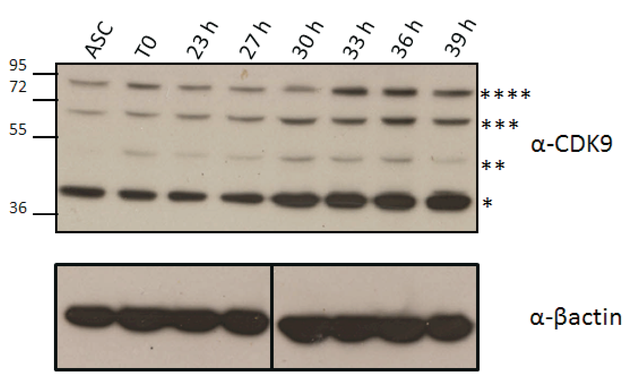

Figure 2: CDK9 expression profile during cell cycle. BJ cells were synchronized in G1 and harvested at indicated time points after realease (time-course is depicted). CDK9 isoforms profile pattern was evaluated by WB (upper panel); β-actin levels were also assessed by WB (lower panel). ASC: assynchronized cells. (*)CDK9 42k; (**) CDK9 55k; (***) and (****) are possibly modified CDK9 bands.

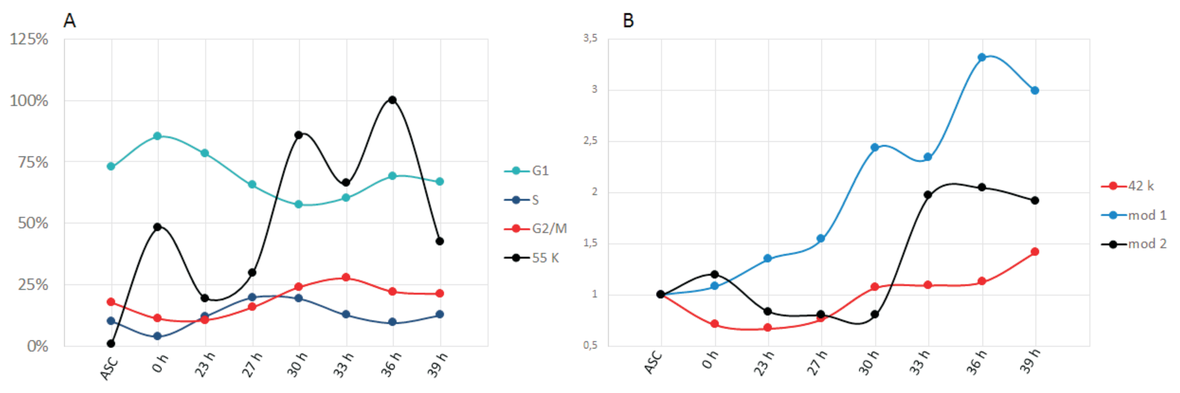

Figure 3: CDK9 expression profile throughout cell cycle. (**A**) 55k evaluation throughout cell cycle analysis (PI staining and flow cytometry analysis) using synchronized BJ cells; colored lines indicate populations in different cell cycle phases. CDK9 levels were assessed by densitometry analysis derived from Figure 2 WB (black line). (**B**) 42k evaluation throughout cell cycle analysis in synchronized BJ cells; colored lines indicate CDK9 42k and putative post-translation modified forms.

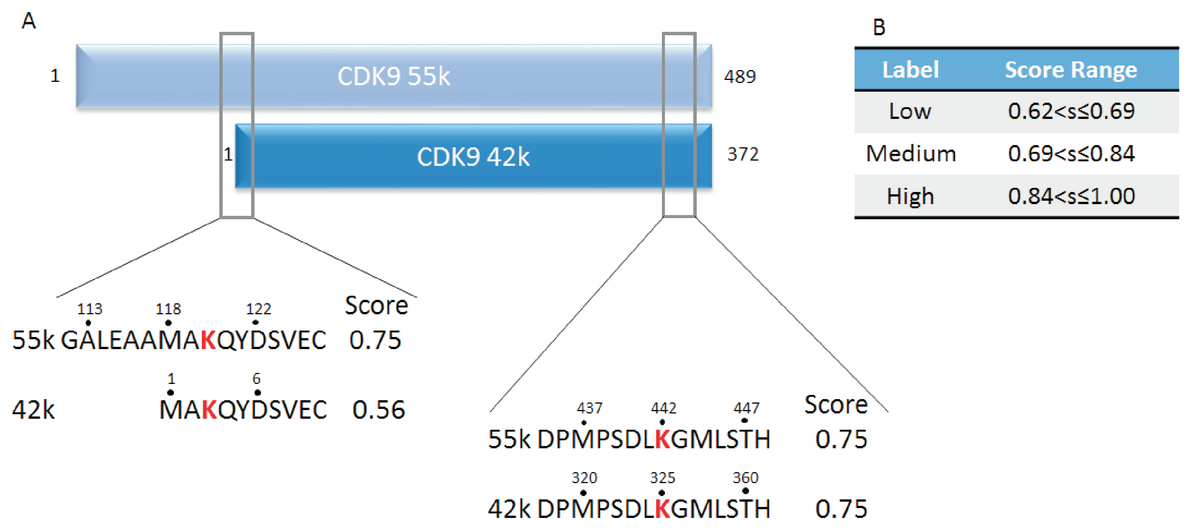

Figure 3: *In silico* analysis of ubiquitinated motifs. (**A**) CDK9 55k and 42k sequences were submitted to UbiPred algorithm, to predict possible ubiquitilation sites. This analysis revelead two ubiquitilation motifs, one presente in both proteins at the C-terminal region (0.75, medium confidence)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

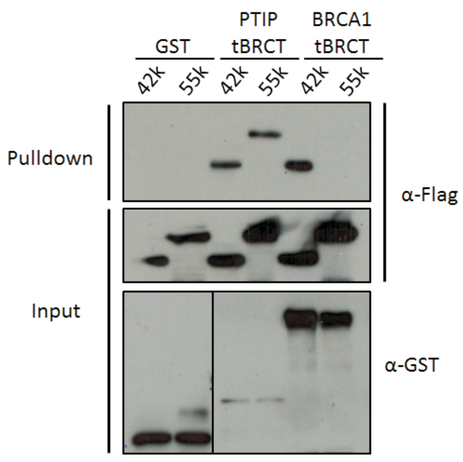

Figure 4: CDK9 interacts PTIP tBRCT. HEK293FT cells were co-transfected with CDK9 42k or CDK9 55k Flag-tagged and A GST construct (empty vector, PTIP tBRCT 5/6 or BRCA1 tBRCT). Cells were harvested 24 hours after transfection and extracts were used in pulldown assay. Upper panel shows CDK9 42k and 55k interaction with PTIP tBRCT and CDK9 42k interaction with BRCA1 tBRCT.

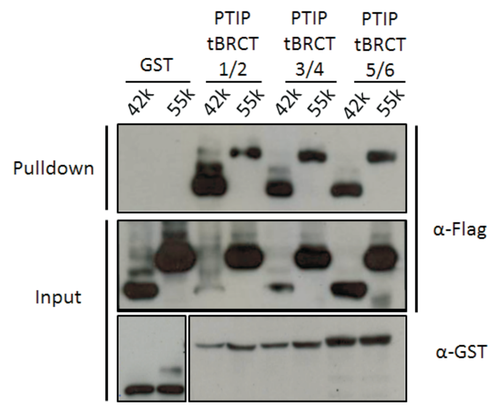

Figure 5: CDK9 interacts with PTIP tBRCTs. HEK293FT cells were co-transfected with CDK9 42k or CDK9 55k Flag-tagged and a GST construct (empty vector, PTIP tBRCT - 1/2, 3/4 or 5/6). Cells were harvested 24 hours after transfection and extracts were used in pulldown assay. Top panel shows both isoforms interacting with all PTIP tBRCTs.

CONCLUSION PERSPECTIVES

To verify whether CDK9 55k is being regulated at transcriptional level, we intend to check CDK9 55k RNA levels during cell cycle.

To better understand the role CDK9 55k in DDR, especially in NHEJ repair, we are generating a human cell line silenced for the 55k isoform using CRISPR/Cas9 technology, targeting exclusively CDK9 55k promoter without affecting CDK9 42k expression.

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

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