Structural and functional characterization of BARD1/CDK13 interaction

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INTRODUCTION

DNA damage response (DDR) is crucial for cell survivor through genomic integrity maintenance. Dysfunctions in proteins implicated in DDR prompt cell to diseases, such as cancer. BRCA1 is a central protein involved in DDR and once damaged plays a pivotal role in cancer development. BRCA1 structure comprises two major domains, a RING finger domain and two tandem BRCT domains (tBRCT). This structure is shared by BRCA1 major interaction partner: BARD1. The tBRCT domain is commonly found in DDR-associated proteins. To study interactions in DDR our group performed a tBRCT interactome for 7 different proteins, using three different strategies: tandem affinity purification followed by mass spectrometry (TAP-MS), yeast two hybrid (Y2H) screening and literature curation. CDK13 was identified as a putative BARD1 tBRCT interactor by both TAP-MS and Y2H strategies. CDK13 is described involved in several processes, such as chromatin remodeling, transcription regulation and splicing coordination, however its biological role remains unclear.

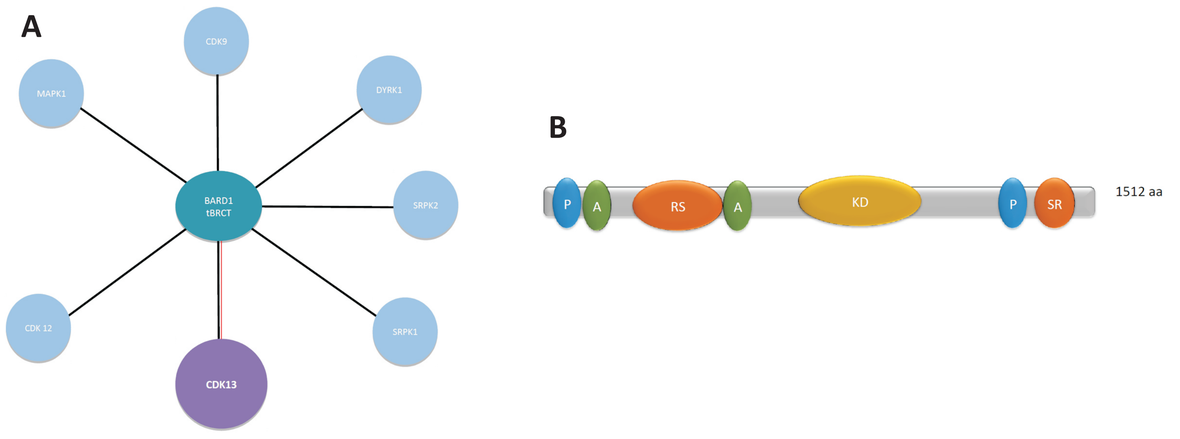


Figure 1 – (A) BARD1 tBRCT kinase likely interactors (Woods et al, 2012). Several kinases were observed interacting with BARD1 C-terminal region throught TAP-MS. Seven diferent kinases were identified. CDK13 (purple circle), unlikely the others, was also collected through Y2H screening. **(B) CDK13 Structure.** CDK13 has serine/arginine (SR), proline (P) and alanine (A) rich regions. This regions may interact with RNA processor proteins. Centrally, CDK13, displays the kinase domain (KD). This domain allows CDK13 act as a kinase protein.

MATERIAL AND METHODS

To confirm BARD1/CDK13 interaction, whole cell extracts obtained from MCF-7 cells were used in co-immunoprecipitation assays using anti-CDK13 and anti-BARD1 . Constructs encoding the HA-tagged regions 1-706aa (N-terminal), 706-982aa (Kinase domain) and 1006-1452aa (C-terminal) of CDK13 were ectopically co-expressed with Flag-tagged full-length BARD1 or GFP-tagged BARD1 tBRCT in Hek293Tcells; whole cellular extracts were used in co-immunoprecipitation assays. CDK13 silencing was performed using lentiviral constructs enconding five different short harpin RNA (shRNA); MCF-7 cells were transduced and extracts were used to determined CDK13 expression profile.

RESULTS AND CONCLUSION

BARD1 and CDK13 interaction was confirmed both in ectopic and constitutive expression approaches. Our data suggest that BARD1 tBRCT and the CDK13 N-terminal region, as well as the kinase domain are critical for the formation of BARD1/CDK13 complex. MCF-7 cells transduced with CDK13 shRNAs lentivirus were submitted to puromycin selection and the cellular extracts were evaluated by immunoblotting to evaluate CDK13 silencing. Cells lacking CDK13 expression will be used to investigate the kinase role during DDR.

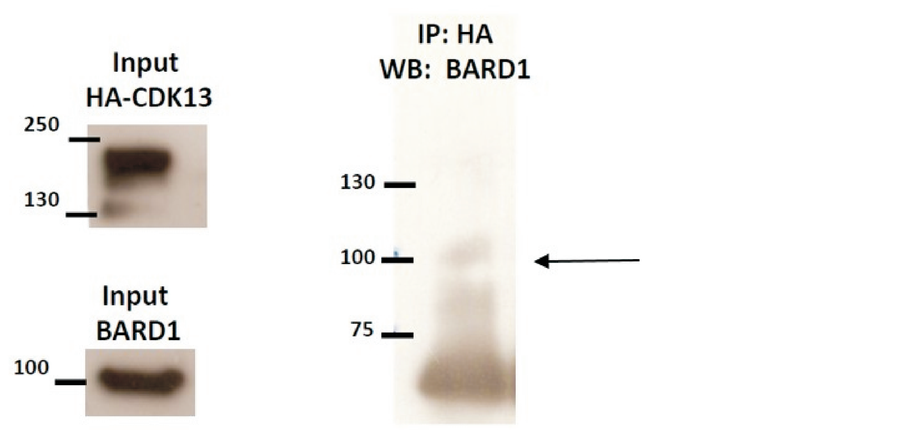


Figure 2 – CDK13 interacts with BARD1. Whole cell extracts (WCE) were obtained from HEK293FT cells transfected with HA-CDK13, coding full-lenght protein (FL), and were used in coimunoprecipitation assays. HA-CDK13 ectopic levels (left upper panel) and BARD1 constitutive levels (left lower panel) were assessed. WCE were precipitated using anti-HA and anti-BARD1 were used in western blot analysis (right panel). The arrow indicates BARD1.

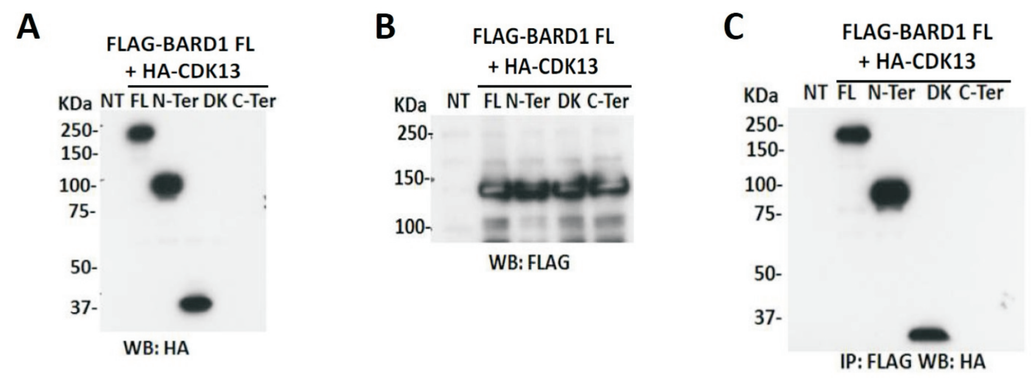
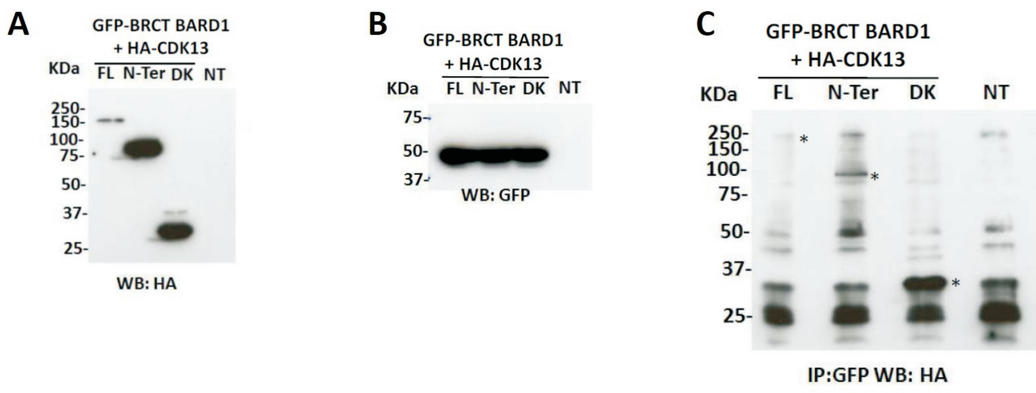


Figure 3 – CDK13 and BARD1 interaction: mapping CDK13 interaction region.

Whole cell extracts (WCE) were obtained from HEK293FT cells cotransfected with HA-CDK13, coding full-lenght protein (FL), or N-terminal region (N-Ter, aminoacids 1-706), or kinase domain (DK, aminoacids 706-982), or C-terminal region (C-ter, aminoacids 1006-1512) and BARD1 fusioned to Flag. WCE were used in coimunoprecipitation assays. HA-CDK13 (Fl, N-ter, DK ou C-ter) ectopic levels are demonstrated in **A** and Flag-BARD1 protein levels are demonstrated in **B**. WCE were precipitated using anti-Flag and anti-HA were used in western blot analysis (**C**).



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