# Characterization of CDK9/cyclin (T1, T2 and K) complexes in the DNA damage response

**Vitória F. Castro[1], Thales C. Nepomuceno[1], Guilherme Suarez-Kurtz[1], Alvaro N. A. Monteiro[2], Marcelo A. Carvalho[1]**

1 Instituto Nacional de Câncer – Divisão de pesquisa clínica

2 Moffitt Cancer Center - Cancer Epidemiology Program

## INTRODUCTION

Cancer is a major health problem worldwide. Tumor biology complexity arises from the accumulation of genetic changes resulting in aberrant regulation of different cellular processes. The DNA damage response (DDR) is a hierarchical multi-step pathway responsible for maintaining genomic integrity. DDR acts by arresting the cell cycle, repairing the damaged DNA and when necessary, activating cellular death programs. DNA double-strand break (DSB) is considered the most toxic type of DNA insult and is responsible for the activation of the ATM-CHK2 DDR axis, necessary for the cell cycle arrest. Further, DSBs can be repaired mostly by two pathways: the error-prone non-homologous end-joining (NHEJ) and the error-free homologous recombination (HR). The cellular choice between NHEJ and HR is modulated by the recruitment of 53BP1 and BRCA1, respectively. Recently, our group characterized the cyclin-dependent kinase 9 (CDK9) as a BRCA1-interacting protein. CDK9 was shown to be necessary for the proper recruitment of BRCA1 to DSB sites, and consequently for HR repair efficiency. CDK9 role as a kinase is dependent on its association with cyclins T (T1 or T2) or K; however, cyclins role in DDR is not clear. The main goal of this project is to characterize which CDK9-cyclin (T and/or K) complex is involved in the modulation of CDK9 in the DDR context.

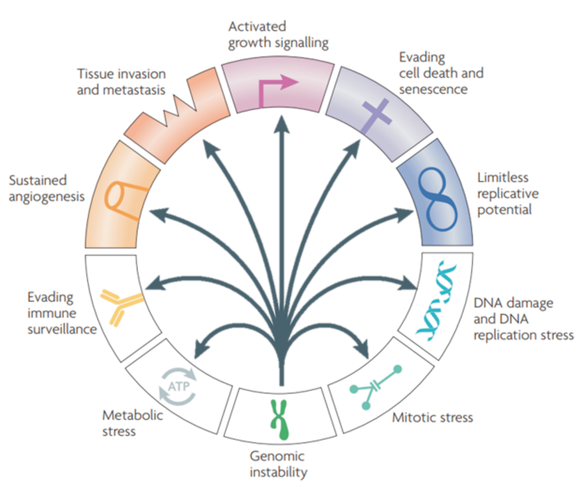



**Figure 1: Genomic instability (GIN) as an enabling hallmark of cancer.** The GIN is characterized as the accumulation of genetic alterations in a cell lifespan. This phenotype can induce the acquisition of all cancer-related characteristics. Adapted from Negrini S. et al., 2010.

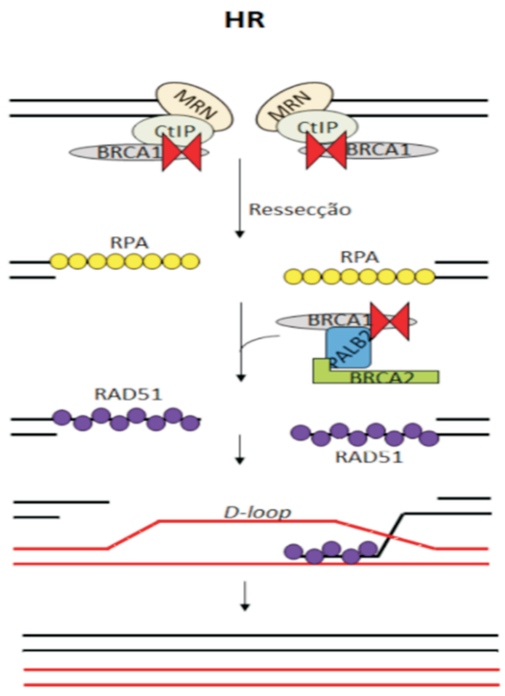
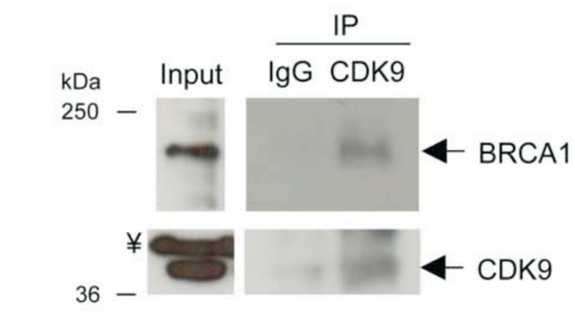



**Figure 2: Homologous recombination (HR) repair pathway.** DNA double-strand breaks are recognized by the MRN complex, which recruits CtIP and BRCA1 promoting the DNA 5' end resection. The single strand DNA generated is rapidly capped by the RPA complex. Further, the BRCA1-PALB2-BRCA2 complex stimulates the substitution of RPA by the recombinase RAD51, which is responsible for the strand invasion on the sister chromatid and consequently error-free repair. Adapted from Nepomuceno, T. 2016.



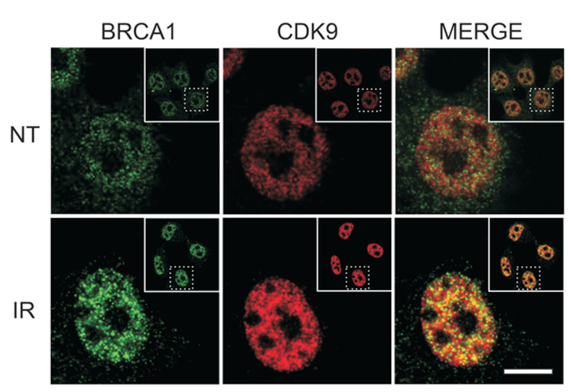




**Figure 3: CDK9 is a DNA damage response (DDR)-related protein.** (**A**) Protein levels were determined in HeLa nuclear extracts by immunoblotting using specific antibodies. Co-immunoprecipitation assays were performed using anti-CDK9 or anti-HA (IgG) antibodies, immunoblots were developed using anti-CDK9 and anti-BRCA1 or BARD1 antibodies, as indicated. ¥ indicate a non-specific band. (**B**) MCF7 cells were exposed to IR (10 Gy) and recovered for 3 hours. Immunostaining was performed using anti-CDK9 and anti-BRCA1 antibodies. Insets depict the nucleus in lower magnification. (upper inset). NT, not treated. Scale bars =10 mm.

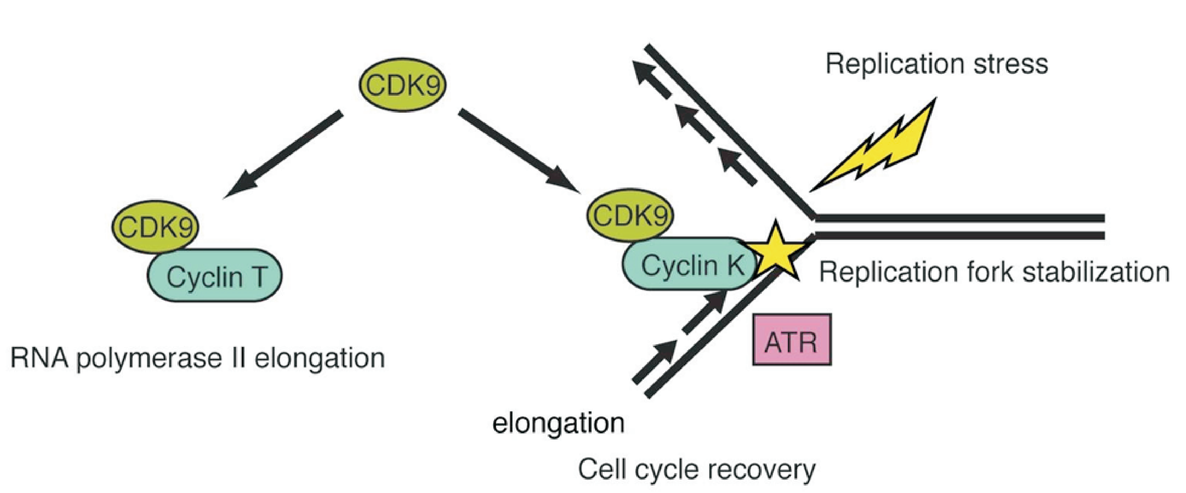



**Figure 4: Proposed model for CDK9-Cyclin T or K role in transcription and cell cycle regulation**. CDK9 acts with Cyclin T on the RNA polymerase II phosphorylation during the transcription elongation step. The CDK9-Cyclin K complex is responsible for maintaining the replication fork stability and promoting the cell cycle recovery after replication stress. Adapted from Yu, D. et al. 2011.

## METHODS AND RESULTS

We analyzed by GST pulldown assays, the interaction of cyclin K with the known DDR-related partners of CDK9, BRCA1 and CHK2. Our data indicate that cyclin K is not capable of interacting with both proteins in the absence of DSBs. We are currently expanding our model to evaluate the interaction between cyclin T (T1 and T2) and these proteins. We are also investigating the impact of DNA damage in these interactions

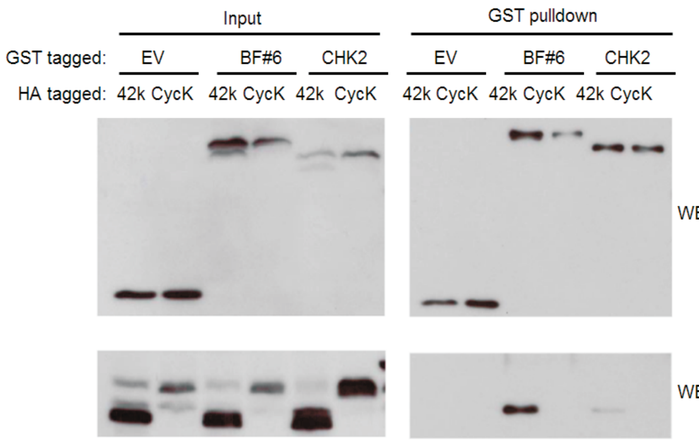



**Figure 5. Cyclin K does not interact with BRCA1 tBRCT and CHK2.** (**Left panels**) co-expression of GST-tagged proteins and HA-tagged CDK9 (42k) or Cyclin K (CycK) in HEK293FT cells. (**Right panels**) GST pull-down assay, Western blots (WB) were developed using indicated antibodies. GST-BF#6 encodes the BRCA1 C-terminal region (from amino acid 1314 to 1863)

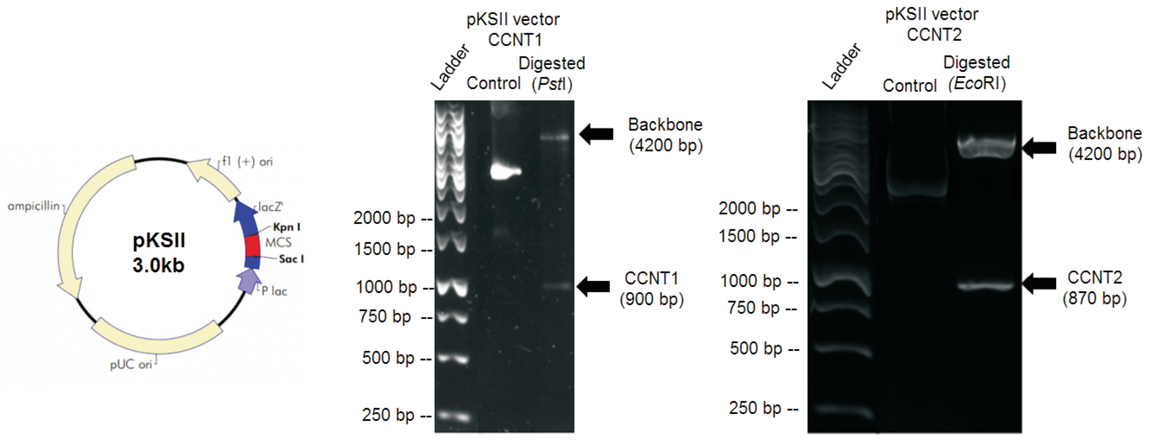



**Figure 6: Restriction analysis of pKSII-CCNT1 and CCNT2 constructs.** (A) pKSII plasmid map. (B) The cloning confirmation was conducted by the indicated DNA restrictions. DNA digestion products were resolved in 1.0% w/v agarose gels. (left panel) pKSII-CCNT1 restriction with PstI. (right panel) pKSII-CCNT2 restriction with EcoRI. The arrows indicate the expected product and their molecular weight. Commercial ladder: GeneRuler 1kb DNA ladder (Thermo Fisher Scientific).

## CONCLUSION

By unrevealing the role of cyclins T and K in the DDR context it will help the understanding of how CDK9 modulates the recruitment of BRCA1 and consequently the DNA damage repair by HR.

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

MINISTÉRIO DA SAÚDE