LABORATORY OF

CANCER CELL BIOLOGY

Genome instability, cell cycle checkpoint, replication stress, protein phosphorylation, cancer, cancer predisposition

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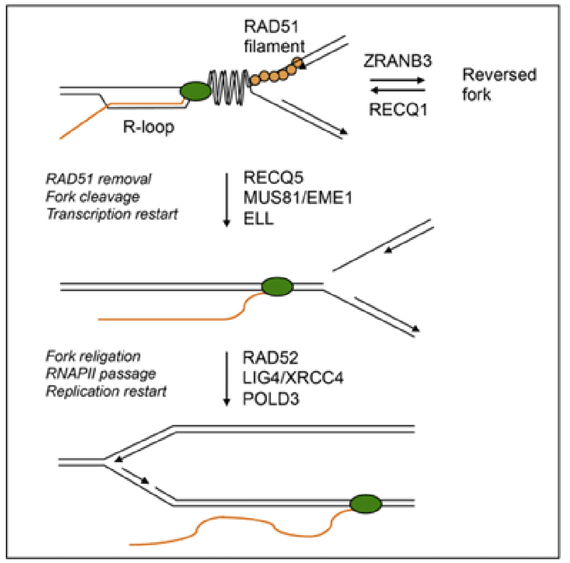
Integrity of human genome is protected by surveillance mechanisms that coordinate the cell cycle progression and DNA repair. In the presence of DNA damage, cells temporarily arrest in the cell cycle checkpoint to prevent transmission of mutations to progeny and continue proliferation after completing DNA repair. The checkpoint and DNA repair are tightly interconnected by signalling cascades that involve protein phosphorylation (ATM, ATR, CHK1/2, CDK1, PLK1 kinases), ubiquitination (BRCA1, RNF168, 53BP1) and gene expression (tumour suppressor protein p53). Deficient cell cycle checkpoints or impaired DNA repair allow proliferation in the presence of damaged DNA, promoting genome instability and eventually malignant transformation. In our laboratory, we employ cell and molecular biology approaches, CRISPR-mediated gene editing and transgenic mouse models to investigate how cells respond to DNA damage. We also seek for genetic defects in cancer cells that could be exploited for personalized cancer treatment.



Replication stress induces R-loop formation in the cell nucleus.

Topic 1. Role of PPM1D/WIP1 in DNA damage response and oncogenesis

Protein phosphatase PPM1D/Wip1 is an important negative regulator of tumour suppressor p53 and promotes termination of the cell cycle checkpoint. High expression of PPM1D/Wip1 is common in cancer. We identified new truncating mutations in PPM1D/Wip1 that impair the cell cycle checkpoints. Using a transgenic mouse model, we have now confirmed the ability of PPM1D/Wip1 mutations to promote cancer. By combining proteomic approaches, biochemistry and cell/molecular biology, we investigate mechanisms of PPM1D/Wip1 function in human cells and seek for its novel targets at chromatin.

Topic 2. Role of R-loops in genomic instability

R-loops are three-stranded nucleic acid structures generated by invasion of the nascent transcript to the DNA duplex behind the transcription complex. R-loops are emerging as a major source of DNA replication stress and genomic instability. We apply mass proteomic approaches and functional siRNA screens to identify novel factors involved in the metabolism of R-loops and G4 structures and study their relationship to DNA replication. Using these approaches, we have recently identified helicase DDX17 as a new factor involved in resolution of the R-loop-mediated transcription-replication conflicts. We have also described a model for resumption of DNA synthesis after fork stalling that requires cleavage by MUS81, ligation by LIG4 and active transcription by elongation factor ELL.

Topic 3. New proteins involved in the cell cycle and mitosis

By expression profiling in human cells, we have identified several new regulators of the cell cycle and mitosis. We found that depletion of FAM110A impaired chromosomal alignment in mitosis and resulted in chromosomal defects. FAM110A localizes at poles of the mitotic spindle and its function depends on phosphorylation by casein kinase 1.

Topic 4. Identification of new cancer-predisposing genes

DNA repair and checkpoint genes are typical tumour suppressors that are commonly inactivated in human cancers. When present in the germline, these mutations increase a risk of cancer development in affected families (such as BRCA1 or CHEK2 in familial breast cancer). In collaboration with medical geneticists, we develop cell-based assays for functional evaluation of newly identified mutations which will allow better prevention of familial cancers.

Selected publications:

1. Martinikova AS, Burocziova M, Stoyanov M, Macurek L*. Truncated PPM1D Prevents Apoptosis in the Murine Thymus and Promotes Ionizing Radiation-Induced Lymphoma. Cells 2020; 9(9):2068.
2. Aquino Perez C, Burocziova M, Jenikova G, Macurek L*. CK1-mediated phosphorylation of FAM110A promotes its interaction with mitotic spindle and controls chromosomal alignment. EMBO Rep. 2021; 22(7):e51847
3. Chappidi N, Nascakova Z, Boleslavska B, Zellweger R, Isik E, Andrs M, Menon S, Dobrovolna J, Balbo Pogliano C, Matos J, Porro A, Lopes M, Janscak P*. Fork Cleavage-Religation Cycle and Active Transcription Mediate Replication Restart after Fork Stalling at Co-transcriptional R-Loops. Mol Cell. 2020; 77(3):528-541.e8.
4. Boleslavska B, Oravetzova A, Shukla K, Nascakova Z, Ibini ON, Hasanova Z, Andrs M, Kanagaraj R, Dobrovolna J, Janscak P*. DDX17 helicase promotes resolution of R-loop-mediated transcription-replication conflicts in human cells. Nucleic Acids Res. 2022; 50(21):12274-12290.