# Laboratory of Genome Integrity

DNA damage response, inflammatory cytokines, cellular senescence, RecQ helicases, R-loops


## Jiří Bartek

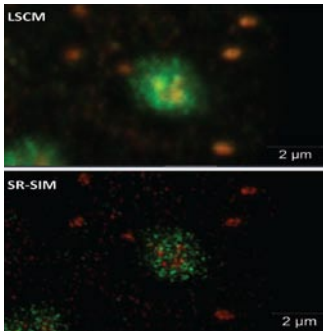
jiri.bartek@img.cas.cz

www.img.cas.cz/research-groups/jiri-bartek


Realization of complex tasks of living organisms depends on the information stored in DNA of their genomes. The loss of this information due to endogenous and exogenous physicochemical damage to DNA results in disintegration of homeostasis at the cellular and organism levels manifested as diseases, including cancer and ageing. Several tightly orchestrated mechanisms take care of preserving the intactness of genetic information by preventing and repairing DNA damage. Our research is centred on cellular responses (collectively termed DNA damage response; DDR) to DNA double-strand breaks, presumably the most deleterious lesions affecting DNA. Cells with unhealed chromosomal breaks are mostly prevented from cell division due to activated DNA damage cell cycle checkpoints; however, following unscheduled cell division, unrepaired breaks result in chromosomal instability with accompanying changes in gene dosage – the driving force of malignant transformation. Specifically, we focus on 1) posttranslational modifications (phosphorylation, ubiquitylation, sumoylation and PARylation) of key players involved in sensing and transmitting signals from DNA breaks to cellular effectors involved in activation of cell cycle checkpoints, DNA repair and cell reprogramming; 2) mechanisms of radioresistance and chemoresistance of cancer cells; 3) mechanisms of cellular response to persistent irreparable DNA damage lesions manifested as irreversible cell cycle arrest (cellular senescence); 4) role of DNA damage-induced expression of secreted factors (cytokines) in autocrine/paracrine signalling, cancer microenvironment and cell reprogramming; 5) exact DNA transactions mediated by RecQ DNA helicases, key players in the maintenance of genomic stability; and 6) impact of the above mechanisms on cancer and ageing with the aim to find new therapeutic approaches, such as thermotherapy using targeted gold nanoparticles.

To summarize our main recent findings, we have identified specific cytokines (IL1β and TGFβ) responsible for genotoxic effects observed in so-called bystander senescence and a mechanism of their action (oxidative stress mediated by elevated expression of NADPH oxidases and downregulation of mitochondrial ATP/ADP translocase type II). We have identified signalling pathways (MAPK and Akt) responsible for radio- and chemo-resistance of prostate cancer cells. Recently, we started a new project aiming to study the molecular mechanisms underlying formation and resolution of RNA:DNA hybrids (R-loops), highly genotoxic structures that can arise as a consequence of collisions between replication and transcription machineries. We focused on identification of proteins associated with R-loops under the conditions of chemically- and oncogene-induced replication stress and studying their role in maintenance of genome stability.

**Fig. 1.** Super-resolution microscopy of DNA damage lesion. Comparison of DNA lesion, marked by repair protein 53BP1 (green) with juxtaposed/co-associated PML nuclear body (red), persisting in human fibroblasts 6 days after ionizing irradiation (10 Gy) acquired by structured illumination microscopy (SR-SIM, bottom) and conventional/laser scanning confocal microscopy (LCSM) image (up).



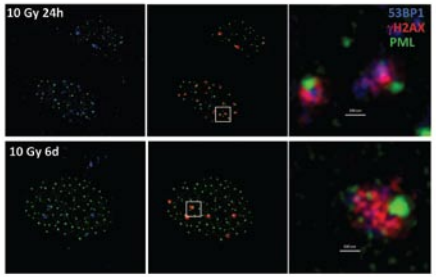




**Fig. 2.** Super-resolution images of DNA lesions persisting in human fibroblasts 1 (up) and 6 (bottom) days after 10 Gy and marked by established DDR markers gH2AX (red) and 53BP1 (blue) co-associated with PML nuclear body (green).


- GACR, GAP305/10/0281 – Role of the Rothmund-Thomson syndrome gene product in maintenance of genomic stability, 2010-2014, P. Janščák
- GACR, GA13-17658S – Mechanisms of radioresistance of prostate cancer cells, 2013-2016, Z. Hodný
- GACR, GA13-17555S – Premature cellular senescence: Mechanisms and links with cancer, 2013-2016, J. Bártek
- MH, NT14174 – The role of 5-azacytidine in immunoepigenetics and genotoxic stress in the treatment of myelodysplastic syndrome., 2013-2015, J. Bártek
- GACR, 14-05743S – Molecular mechanism of genomic instability caused by oncogene activation, 2014-2016, P. Janščák
- MEYS, LH14037 – Identification of protein complexes associated with genotoxic RNA: DNA hybrids and their role in maintenance of genomic stability, 2014-2016, J. Dobrovolná
- GACR, GA204/09/0565 – Role of RECQ5 DNA helicase in maintenance of genomic stability, 2009-2013, P. Janščák
- GACR, GPP305/11/P683 – Post-translational modifications of Daxx and their functional relevance in DNA damage response and cellular senescence, 2011-2013, H. Hanzlíková



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**From the top left:** Rastislav Dzijak, PhD / Postdoctoral Fellow, Jana Dobrovolná, PhD / Research Associate, Soňa Hubáčková, PhD / Postdoctoral Fellow (since 2013), Blanka Gabajová, MSc / PhD Student (since 2013), Hana Hanzlíková, PhD / Research Associate (until 2014), Václav Urban, MSc / PhD Student Terezie Imrichová, MSc / PhD Student (since 2013), Kateřina Krejčíková, MSc / Research Assistant, Michal Šrámek / Technician (since 2013), Monika Žárská, MSc / Research Assistant, Eliška Davidová / Diploma Student, Markéta Vančurová , MSc / Research Assistant, Alena Kučerová / Diploma Student (since 2014) Lucie Knoblochová / Diploma Student (since 2013), Lenka Kyjacová, MSc / PhD Student, Edita Křížová, MSc / PhD Student (since 2014), Zdeněk Hodný, MD, PhD / Deputy Head

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