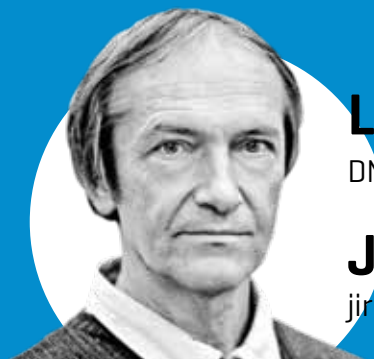

Laboratory of Genome Integrity

DNA damage response, cell cycle, oncogenic transformation, cellular senescence, ageing

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Realization of complex tasks of living organisms depends on information stored in DNA of their genomes. The loss of this information due to endogenous and exogenous physicochemical damage of DNA results in disintegration of homeostasis at the cellular and organism level manifested as diseases including cancer and ageing. Several tightly orchestrated mechanisms take care to preserve the intactness of genetic information by preventing and repairing DNA damage. Our research is centered on cellular responses (termed collectively 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 acetylation) of key players involved in sensing and transmitting signals from DNA breaks to cellular effectors responsible for activation of cell cycle checkpoints and repair; 2) mechanisms of DNA damage cell cycle checkpoint recovery after successful DNA repair; 3) mechanisms of cellular response to chronic irreparable DNA damage manifested as irreversible cell cycle arrest (cellular senescence); and 4) role of DNA damage-activated expression of secreted factors (cytokines) in autocrine/paracrine signalling and intercellular communication, and 5) impact of the above mechanisms on cancer and ageing.

Recently, we have characterized cytokine expression in chemotherapeutic drug-induced cellular senescence and the role of activated interferon JAK/STAT signalling pathway in autocrine/paracrine induction of tumour suppressors such as PML, STAT1 and IRF-1. We have found that normal and tumour human cells escaping acute intoxication with specific bacterial toxin, cytolethal distending toxin, undergo irreversible cell cycle arrest with all hallmarks of cellular senescence including irreparable chromosomal damage and cytokine expression.

In collaboration with R. Medema group (University of Utrecht, Netherlands), we have investigated the role of Wip1 phosphatase in dephosphorylation of DNA damage-associated modification of histone protein H2AX and in DNA damage checkpoint recovery. Currently, we are performing high-throughput siRNA-based phenotypic screening to discover factors involved in posttranslational modifications of DDR components and functional characterization of positive 'hits'.

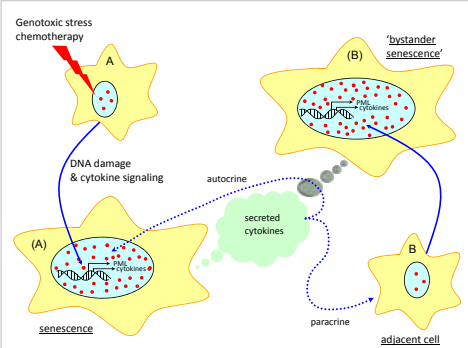

Fig. 1. Model of PML and cytokine signalling in drug-induced and 'bystander' senescence. Cytokine secretion and autocrine/paracrine signalling triggered by the DDR machinery upregulate PML expression and formation of PML NBs, collectively leading to cellular senescence, both directly and through 'bystander' effects.

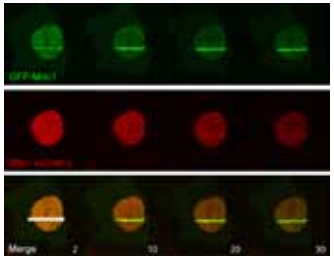

Fig. 2. Wip1 phosphatase translocates to sites of DNA damage. U2OS cells transfected with MDC1-GFP and Wip1-mCherry plasmids were grown in glass-bottom wells in a permanently heated chamber and were presensitized by Hoechst-33342 (0.5 μg/ml) treatment for 1 h. DNA damage was induced by microirradiation of indicated regions with a 405 nm laser. Images were acquired at the indicated times after microirradiation (min).

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- GA CR, GA301/08/0353 – Protein modifications in DNA damage signalling: Mechanisms and cancer relevance, 2008-2010, J. Bartek
- GA CR, GA204/08/1418 – The role of the JAK/STAT signalling pathway in cellular senescence, 2008-2012, Z. Hodný
- FP7 EU, 223575 TRIREME – Systems-level, multi-layer understanding of cellular responses to ionizing radiation, 2009-2012, J. Bartek
- GA CR, GAP301/10/1525 – Mechanisms of DNA damage checkpoint termination, 2010-2012, J. Bartek
- GA CR, GPP305/10/P420 – Role of Wip1 phosphatase in the DNA damage response, 2010-2012, L. Macůrek

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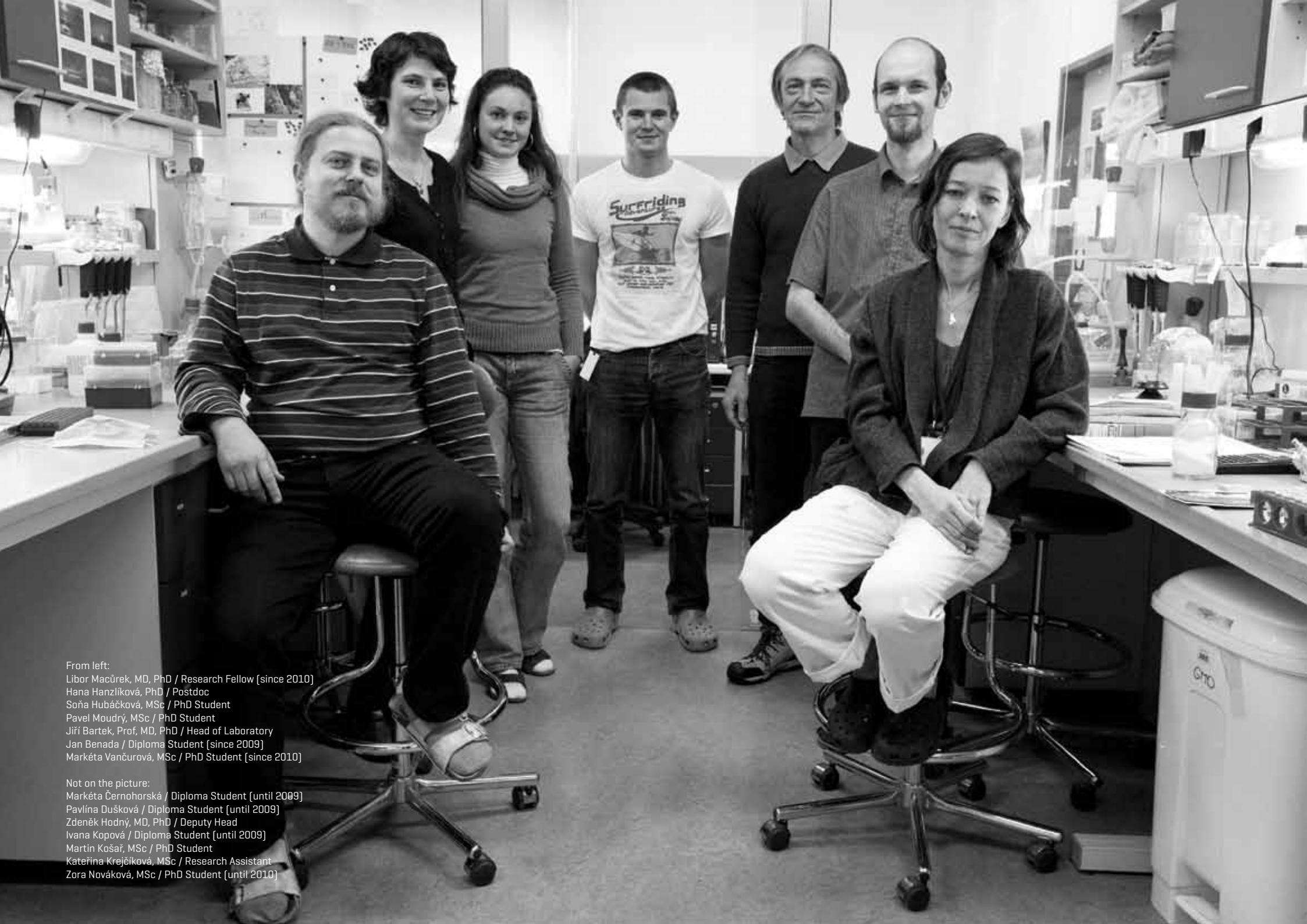

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Hana Hanzlíková, PhD / Postdoc
Soňa Hubáčková, MSc / PhD Student
Pavel Moudrý, MSc / PhD Student
Jiří Bartek, Prof, MD, PhD / Head of Laboratory
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Markéta Černohorská / Diploma Student (until 2009)
Pavlína Dušková / Diploma Student (until 2009)
Zdeněk Hodný, MD, PhD / Deputy Head
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