Abstract

The dissertation deals with a cell response to genotoxic stress, specifically to anti-cancer treatments with a genotoxic mechanism of action. In principle, cells can respond to these perturbing stimuli in several ways: in case of severe DNA damage, they usually undergo apoptosis or enter senescence. In case of minor DNA damage, or upon defective checkpoint mechanisms, they may continue the cell cycle, either with successfully repaired DNA or with mutations of various kind. Thanks to selection pressure, the mutations that provide cells with a certain growth advantage under conditions of continuing genotoxic stress, gradually accumulate and render the tumor treatment-resistant. In my thesis, I focus on several aspects of this whole process.

First, I participated in a characterization of a radioresistant and anoikis-resistant population of prostate cancer cells. This population was generated by irradiating cells 35 times by 2 Gy, a regime used in clinics. After this treatment, a population of low-adherent cells emerged that demonstrated increased expression of EMT- and stem cell markers. The low-adherent state of these cells was maintained by Snail signaling and their anoikis resistance by ERK1/2 signaling. Interestingly, after a protracted period of time, these cells were able to re-adhere and restart proliferation, while retaining their tumorigenic potential, as demonstrated by their injection into nude mice. Finally, the survival of these cells was compromised by combined AKT and ERK1/2 inhibition.

Second, I took part in a study where the effects of chemotherapy-induced senescent cells on tumor growth was examined. It was found that docetaxel- or radiation-induced senescent cells accelerate tumor growth, when co-injected with normal proliferating cells into mice with non-compromised immune system. Furthermore, this accelerating effect, as well as the growth of the tumor itself, was reverted by IL-12, a cytokine with known immunostimulatory properties.

Third, I cooperated on a project the aim of which was to find a specific marker of senescent cells. The surface protein L1CAM was identified as a promising candidate, as its mRNA and protein levels were increased in a majority of examined cell lines brought into senescence by various stimuli (serial passaging, γ-radiation, BrdU, IFNγ, TGF-β). Furthermore, the expression of the protein was closely connected to the metabolism, as its expression changed upon cell cultivation in a high-glucose medium, after inhibition of the mevalonate pathway or after downregulation of mitochondrial ATP/ADP translocator ANT2. The role of the protein in cell migration and adhesion was also confirmed. Finally, the reciprocal negative

regulation between L1CAM and the ERK1/2 signaling pathways was described, which explained our previous observation that L1CAM levels were not increased upon H-RAS-induced senescence.

In my own project, I attempted to better understand the genotoxic stress-induced association of the PML protein with the nucleolus. PML has been described as a tumor suppressor and a senescence inducer; and nuclear bodies formed by this protein are specifically present in senescent cells. We found out that a combination of RNA polymerase I (RNAPI) inhibition and topological stress leads to the translocation of PML to the nucleolus and we identified the domains of PML that are important for this interaction, while one of them is also necessary for PML association with SUMOylated proteins. Using super-resolution and time-lapse microscopy, we described how the PML nucleolar associations (PNAs) form, how they evolve and what is their 3D structure. Furthermore, we showed that PNAs contain rDNA, they co-localize with SUMO signal and, in their last stage, they accumulate proteins that are involved in rDNA metabolism. Finally, we described the association of PNAs with γH2AX, a marker of damaged DNA. Therefore, we propose a model when combination of RNAPI inhibition and topological stress leads to a specific type of rDNA damage that is followed by attraction of SUMOylated proteins and formation of PNAs. PNAs then participate on a sequestration and processing of damaged rDNA loci. When unresolved, these damaged loci persist in cells and probably contribute to the onset of senescence.