Abstract

Organisms such as mammals need tissue renewal as an important process for maintenance of their viability. Because proliferation is essential also for tumourigenesis, cells need tumour-suppressor mechanisms to protect organism against cancer. Cellular senescence, the permanent state of cell-cycle arrest, features one of these intrinsic barriers against tumourigenesis after DNA damage and understanding of this process may lead to finding of novel therapeutic targets and to optimization of chemotherapy for patients with cancer.

In the first part of the PhD thesis, we investigated activation of JAK/STAT signalling pathway in drug-induced senescence. We used genotoxic drugs like aphidicolin, camptothecine, 5-bromo- 2´-doexyuridin, etoposide or thymidine to induce premature senescence in normal and cancer cells. All this chemicals were able to persistently activate JAK/STAT signalling in monitored cells. Activation of STATs was accompanied with up-regulation of expression of interferon-stimulated genes (ISGs), such as MX1, IRF1, IRF7 and PML. Since IRF1 and IRF7 can be directly involved in stimulation of the IFN genes, we show activated expression as well as secretion of IFNbeta and IFNgamma, but not IFNalpha in drug-induced senescent cells. Furthermore, an inhibition of JAK1 as a major kinase of STAT activation led to suppression of STAT activation and expression of ISGs, supporting a dominant role of JAK1 in activation of interferon response in drug-induced senescent cells. Besides interferons, we found significantly elevated expression and secretion of several other cytokines, like pro-inflammatory IL1, IL6, IL8 and IL10 or TNFalpha. Because JAK/STAT is activated through cytokines (mainly by interferons and IL6), our results suggest a positive feedback loop between JAK/STAT prolonged induction and cytokine secretion.

Promyelocytic leukaemia protein (PML) is one of the well known tumour suppressor upregulated in almost all forms of cellular senescence. It is an essential component of nuclear structures known as PML NB nuclear bodies (NBs) whose accumulation occurs in response to stress. These bodies represent sites where activation of many regulators of cell cycle progression including p53 and Rb proceeds. As we showed, PML NB also colocalize with sites of double strand breaks detected by accumulation of gammaH2AX or 53BP1. In drug-induced senescence, we found that upregulation of PML, a building component of PML NBs belonging to interferon-stimulated genes, is mediated by JAK/STAT pathway and not by p53, as knockdown of JAK

kinase family abolished activation of JAK/STAT pathways as well as PML gene expression and formation of PML NBs in senescent cells in contrast to knockdown of p53, which has no effect on level of PML mRNA and protein. Next we found that transcription of PML is controlled through the Interferon Stimulated Response Element (ISRE) located on its proximal promoter, which is known binding site for STATs, and deletion of this sequence suppressed the transcription efficiency of PML promoter.

The number of PML NBs differs in various cell types cultivated under unperturbed conditions. Therefore we analyzed, whether PML transcription is regulated by JAK/STAT signalling pathway also on its basal level. Interestingly, we found that the number of PML NBs and the levels of PML transcript and protein correlate with activity of STAT3, but not STAT1 or STAT5 in three model cell lines selected for low (U2OS), middle (HeLa) and high PML expression (BJ). Similar correlation was observed between secretion of IL6 - a major activator of STAT3 signalling pathway - and PML NBs levels. Knock-down of STAT3 by specific siRNA as well as a depletion of IL6 from medium by neutralizing antibodies down-regulate PML expression. We found that IL6 alone is able to activate PML transcription in exposed cells. Using chromatin immunoprecipitation, we proved direct binding of STAT3 but not STAT5 to ISRE of PML gene regulatory region.

STAT3 is a main effector activated by IL6, but recent works described IL6-dependent activation of NFkB, a transcription regulator of set of genes involved in immune response, inflammation, proliferation and DNA damage response through IL6/PI3K/Akt signalling pathway. Interestingly, we found that downregulation of NEMO, a molecule important for NFkB activation, also led to downregulation of PML transcription after IL6 treatment.

It is reported that PML NBs play an important role as a docking site for many proteins involved in DNA damage signalling and senescence including those involved in formation of so called „senescence-associated heterochromatin foci“ (SAHF), such as HIRA, HP1 and Rb. While HP1 is well known family of proteins playing role in heterochromatinization, Rb was recently found as the protein involved in formation of SAHF after binding to PML NBs. Elevation of PML NBs was found in all types of senescence, therefore we investigated a presence of SAHF in different types of senescence. We found that all tested cell types formed SAHF under the condition of oncogene-induces senescence but not after treatment with etoposide, doxorubicin, hydroxyurea or bacterial genotoxins where their formation was cell type specific and correlated

with accumulation of p16 in cells. These results indicate that SAHFs are not a common feature of cellular senescence. Since SAHF formation was described to play the role in silencing of genes important for maintenance of cell proliferation, our results show that these structures are not necessary for induction of senescence but if they are present they support development of senescence through activation/stabilization of p16/Rb pathway.