PhD Thesis – ABSTRACT

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The tumor suppressor protein PML and PML nuclear bodies (multiprotein structures characterized by concentrated amount of PML) have been implicated in many cellular processes such as regulation of cell proliferation, stress response (to DNA damage, heat shock, viral infection), apoptosis, and senescence, however their specific function and mechanism of PML expression remain obscure. Chromosomal translocation between the PML gene and the gene for retinoic acid receptor alpha causes aberrant stabilization of repressor complexes including histone deacetylase on promoters of retinoic acid-induced genes and leads to acute promyelocytic leukemia (APL). Trichostatin A (TSA), an inhibitor of histone deacetylases, was successfully used to reactivate suppressed genes in APL cells.

In presented PhD thesis we focused on examination of PML expression in response to interferon signaling with the emphasis on an interference with inhibitors of histone deacetylases. We have shown that interferon alpha (IFNα)-induced expression of PML at mRNA and protein level and subsequent increase of PML nuclear bodies are suppressed by variety histone deacetylase inhibitors (HDACIs) independently of cellular origin, whereas basal (i.e. IFNα-nonstimulated) expression of PML is not influenced by HDACIs. The classical Jak-STAT pathway mediating transition of IFNα signal remained unaffected by HDACIs, and we concluded that the target of histone deacetylases lies downstream of the Stat1-Stat2-IRF9 complex binding to PML promoter. Moreover, we have shown similar inhibitory effect of HDACIs on induction of other interferon-induced genes, Sp100 (a component of PML nuclear bodies) and IRF1. Taken together with other recent studies, we supported the hypothesis that a deacetylation event is necessary for full transcriptional activation of many if not all interferon-induced genes and that HDACIs can block it. HDACIs are currently intensively tested as potent anticancer drugs, and some of them entered advanced phases of clinical trials. Understanding the biological activity of HDACIs will help to predict the long-term effects of these drugs on normal cells and to avoid the potential side effects. Specifically, the effect of HDACIs on IFN pathway seems to be important since it can interfere with physiological functions of IFNs that play a critical role in the cellular antiviral defense and are also widely used for treatment of various cancer types.

In second part of this work we examined the changes in PML expression with respect to level of cell differentiation, proliferative age and in a status of cellular senescence, that serves as an anti-cancer barrier. We have shown that human mesenchymal stem cells (growing, confluent or terminally differentiated) express PML protein, form PML nuclear bodies and respond to IFNα treatment by dramatic elevation of PML mRNA and protein levels and moderate increase of PML NBs. Number of PML NBs per cell nucleus is dependent on the culture proliferative age. In normal diploid but not in immortal cells PML forms various nucleoli-associated structures. The localization of PML into nucleoli is reestablished in senescent cells. The importance of PML association with nucleoli for its tumor suppressor activities is hypothesized. Further, we have demonstrated that drug-induced senescence in several human cell lines leads to increased PML expression and formation of PML NBs. The senescent cells have changed sercetory phenotype and exhibited elevated expression of IFNβ and other cytokines. These cytokines probably through auto/paracrine mechanism activate Jak-STAT signaling pathways resulting in up-regulation of interferon stimulated genes including PML, STAT1, IRF1, IRF7, Mx1, ISG15, 2',5'-oligoadenylate synthetase 1, and others. Observed activation of IRF1 and IRF7 suggests that senescent phenotype, can be sustained in positive feedback loop.

In summary, this work contributed our understanding of the mechanisms regulating the expression of PML gene normal and senescent cells.