Abstract

Epigenetic mechanisms of transcriptional regulation and modulation of gene expression using RNA interference have become a powerfull tool for studying various cellular mechanisms, including tumor progression and viral latency.

Advances in expression profiling have provided technology to detect candidate genes implicated in this complex process and bring other possibilities in the diagnosis and treatment of tumor diseases. In this work, I compare the gene expression profiles of v-*src*-transformed metastatic and nonmetastatic cells using microarray chip technology. Transcription factor homeodomain only protein X (*HOPX*) was identified as one of the differentially expressed genes. Activity of *HOPX* gene in several cancer types is usually controlled by promoter methylation. The role HOPX in metastatic formation was assessed by inoculation of cells with modulated expression of *HOPX* in a syngeneic chicken model system. After *HOPX* knockdown using shRNA, originally metastatic line showed decreased *in vivo* metastatic capacity. Further genomic analyses identified a cadre of genes affected by *HOPX* knockdown. These data demonstrate that *HOPX* is a metastasis-associated gene and that its knockdown decreases the metastatic activity of v-*src*-transformed cells through altered gene expression patterns.

In the second project, I focused on HIV-1 latency. Antiretroviral therapy (ART) effectively suppresses HIV replication, but HIV persists during ART due to long lived and proliferating latently infected CD4+ T-cells in transcriptionally silent but replication competent form. One strategy to eliminate virus HIV-1 is to activate virus transcription using latency reversing agents and potentially trigger cytolysis nebo immune-mediated clearance. CpG methylation accompanied by repressive histon code seems to be the ultimate step in the development of the silent state of the provirus. In this study, I employed cellular clone harboring a latent HIV-1 mini-provirus, whose reactivation can be monitored according to the percentage of EGFP-positive cells. The clone displays a high level of 5´LTR DNA methylation of the first CpG island and the latent provirus is resistant to reactivation. We knocked down the expresssion of DNA methyltransferase 1 and 3B (*DNMT1* and *DNMT3B*) or histon deacetylase 2 (*HDAC2*) by siRNA in model cell line and analyzed the HIV-1 provirus reactivation by NF- κB inducers and 5´LTR DNA methylation level. I

demonstrated that the depletion of DNMT1, but not DNMT3B or HDAC2, contributed to a partial loss of 5´LTR methylation in our model cell line with hypermethylated 5´LTR DNA of the first CpG island.

Our third study is focused on 5´LTR DNA methylation of the endogenous retroviral loci *ERVWE1* encoding human fusogenic glycoprotein *Syncytin-1*, that contribute to the differentiation of placental syncytiotrophoblast. However it was reported, that in seminoma, *Syncytin-1* expression is increased. Seminomas are subtypes of testicular germ cell tumors (TGCT) that emerged from the primordial germ cells, which have undergone a process of global demethylation. I assume that *ERVWE1* derepression could be the direct consequence of the genome hypomethylation in seminoma cells. I therefore explored the levels of 5-methylcytosine (5-mC) and demethylation interemdiate 5-hydoxymethylcytosine (5-hmC) at the *ERVWE1* promoter using bisulfite and oxidative bisulfite sequencing in several samples of seminomas. I conclude that DNA demethylation of the *ERVWE1* promoter in seminomas is a prerequisite for Syncytin-1 derepression.

Key words: metastatic disease, HOPX, v-*src* transformation, HIV-1 latency, antiretroviral therapy, DNA methyltransferase 1, Syncytin-1, 5-methylcytosine, 5-hydroxymethylcytosine, seminomas