The role of *de novo* DNA methyltransferases in transcriptional silencing of retroviruses and retroviral vectors derived from avian sarcoma and leukosis virus.

Abstract

Avian sarcoma and leukosis virus-based (ASLV) vectors are retrovirus-derived vectors that can replicate efficiently in avian cells, but are replication-defective in mammalian cells. ASLV genome integration is nonclustered, does not favor gene-rich regions, transcription start sites, or CpG islands. There was no propensity for ASLV insertions within or near proto-oncogenes. Thus, they are quite safe and advantageous for use in gene therapy and vaccines. One of the obstacles to the use of ASLV-derived vectors in mammalian cells is the transcriptional silencing of integrated proviruses. In general, gradual silencing of transduced vectors correlates with epigenetic changes of retroviral long terminal repeats (LTRs). CpG methylation of DNA and/or modifications of histones in nucleosomes linked to the promoter region were found in silenced proviruses in vitro. The aim of this diploma thesis was to reveal the influence of the de novo DNA methyltransferases (DNMT) on transcriptional silencing of integrated ASLV proviruses in chicken and human cells. We wanted to find out, if mammalian DNMT3a as holoenzyme is able to recognize integrated avian provirus and silence it. For this purpose we used chicken cell line DF-1, where high level of expression of mammalian DNMT3a induced by mammalian inducible system is present. We also wanted to discover whether human de novo DNMT3b has an influence on silencing of ASLV retroviral vector. Therefore we used wild type (WT) and DNMT3b deficient cell lines HCT116 derived from colorectal carcinoma. Clones with silent proviruses were chosen for reactivation by 5-aza-cytidine (5-azaC) and trichostatin A (TSA) to recover the stability and way of proviral silencing. Proviral methylation statuses of these proviruses were disclosed by bisulfide conversion and sequencing. Our results proved that mouse DNMT3a is not able to silence integrated provirus by methylation in chicken cell line DF-1. Our data also reveal that DNMT3b plays very important role in proviral silencing and is essential component of de novo methylation dependent proviral mechanism of silencing. Proviruses in WT clones were efficiently reactivated by 5-azaC and proviruses in clones of DNMT3b deficient cell line were much more efficiently reactivated by TSA.