ENDOGENOUS REVERSE TRANSCRIPTASE (RT) ACTIVITY AND CHROMATIN REMODELING IN NORMAL AND TRANSFORMED CELLS AND EARLY EMBRYOS

Corrado Spadafora (a), Ilaria Sciamanna (a), Tom Misteli (b)
(a) Service of Biotechnology and Animal Welfare, Istituto Superiore di Sanità, Rome, Italy
(b) Cell Biology and Genomes Group, National Cancer Institute, National Institutes of Health, Bethesda, USA

State of the art

Endogenous Reverse Transcriptase (RT) is an enzyme encoded by two classes of genomic retroelements: retrotransposons and endogenous retroviruses. Basal levels of RT are expressed in all non pathological, differentiated tissues while high RT expression levels characterize tumorigenic cells, germ cells and embryonic tissues.

Preliminare studies carried out in our laboratory have shown that RT inhibition using pharmacological inhibitors (nevirapine and efavirenz, two drugs currently used in AIDS therapy) drastically reduces cell proliferation, promotes differenziation of tumorigenic cells *in vitro*, induces a riprogrammed gene expression and antagonizes tumor progression in nude mice inoculated with tumorigenic human cell lines, including melanoma, prostate and colon carcinoma and microcitoma. Discontinuation of the treatment with RT inhibitors resumes the original proliferation level and tumor progression and riestablish the gene expression profile as in non treated cells, thus suggesting that RT plays an epigenetic role in cell differentiation and in tumorigenesis. Moreover, RNA interference essay (RNAi) targeted against the human RT-encoding LINE1 retroelement family, confermed the results obtained with the drug treatment.

Experimental activity carried out in the frame of the collaborative Project ISS-NIH

Results obtained in previous years revealed some key functional aspects which contributed to define the role of RT in tumorigenesis. We have observed that exposure of tumorigenic cells to RT inhibitors causes a global remodeling of chromatin which involves not only specific genes, whose expression is modulated upon drugs treatment, but also genome districts that remain usually silent, as centromers and heterochromatin. More specifically, we have shown that nevirapine treatment induces an increased expression of specific heterochromatin markers and enhances the amount of heterochromatin in the nuclei of tumorigenic cells as compared to non-treated control cells. Changes in chromatin organization which follow the treatment with nevirapine, are concomitantly associated with an ample modulation of gene expression, as emerged from microarray analysis carried out in human melanoma cells. We have found that the treatment with nevirapine or efavirenz, induces a riprogrammed expression of 159 and 92 genes, respectively. More in details, 90 genes are up-modulated and 69 down-modulated with nevirapine while 58 genes are up-modulated and 34 down modulated with efavirenz. A group of 30 genes are equally modulated with both drugs. Gene Onthology classification of the

modulated genes showed that specific classes of genes are preferentially modulated upon drug treatment:

- morphogenesis, from 7.8% in control cells to 27.27% in treated cells
- development, from 5.7% in control cells to 18.2% in treated cells
- cell proliferation, from 1.48% in control cells to 8 % in treated cells

In order to extend this analysis and to achieve a deeper knowledge of the process of reprogramming gene expression, we have comparatively analyzed the gene expression profile of microRNA in melanoma cells treated with nevirapine and non-treated. We have identified 20 microRNA whose expression is significantly modulatedf: 17 miRNA are down-modulated while three are up-modulated following nevirapine treatment. The vast majority of modulated microRNAs regulate the expression of oncogenes and of tumor suppressor genes implicated in the genesis of a variety of tumors, including melanoma.

As a whole, these results confirm the original hypothesis that: i) endogenous RT plays a key role in the mechanism controlling global gene expression and ii) that selected classes of genes and microRNA, implicated in development and cell proliferation processes, are preferential targets of RT.

Mechanism and model

The present results, together with those in previous years of the ISS-NIH Program, allowed us to develop a possible RT-based mechanism controlling the global expression of RNA, both coding and non-coding. RT inhibition causes essentially three main consequences in tumor cells: i) chromatin rimodeling; ii) alteration of mRNA gene expression profile, and iii) alteration of miRNA gene expression profile. These data, together with the recent identification of the target substrate of RT, that catalizes the formation of a hybrid DNA/RNA structure in tumor cells, suggested a RT-mediated gene expression regulatory mechanism. Briefly, endogenous RT, highly active in cells with a high proliferative potential, reverse transcribes RNA molecules creating DNA/RNA hybrid structures and antagonizing the formation of double-stranded RNA. The suppression of double-stranded RNA inhibits the subsequent formation and maturation of miRNAs. Under these conditions, cells are maintained in a poorly differentiated, highly proliferating state. A preliminary confirmation of this model was the finding of DNA/RNA hybrid structures in tumorigenic cell lines cellule but not in normal cell lines or in tumorigenic cell lines exposed in vitro to RT inhibitors.

Work is currently in progress aiming at confirming this model.

Some of the experimental work reported and the development of the model are derived from the fruitful collaboration that was developed in these years between our laboratory and the laboratory of Dr. Misteli at NCI/NIH.

Publications of the project

Sciamanna I, Oricchio E, Limongi MZ, Prosseda G, Misteli T, Spadafora C. “A Reverse Transcriptase-dependent mechanism regulates gene expression and chromatin organization in cancer cells”. Manuscript in preparation.

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Sciamanna I, Oricchio E, Pittoggi C, Beraldi R, Spadafora C. Functional roles of a reverse transcriptase-dependent machinery in embryonic development and cell transformation In: *Cold Spring Harbour meeting Dynamic Organization of Nuclear Function*; Cold Spring Harbor, NY, 17-21 September 2008.

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