Transcription factor REST does not affect acetylation of H3 histone induced by Valproic Acid in HeLa cells

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Defined regulation of gene transcription is extremely important for cell function and its disturbance results in developmental defects that could lead to certain types of cancer. Transcription factors are essential for the regulation of gene expression. Another important process that induces or represses transcription is epigenetic control that includes the post-translational modification of histones, DNA methylation and mRNA or protein degradation regulated by non-coding RNAs (siRNA, miRNA).

The transcriptional repressor REST (RE1 silencing transcription factor, also known as NRSF – neuron-restrictive silencing factor) binds to a conserved RE1 motif and represses many neuronal genes and several microRNAs in non-neuronal cells. REST regulates its target genes' expression through recruitment of nucleosome-modifying enzymes to RE1 sites, including histone deacetylases (for example HDAC1/2), demethylases (for example LSD1), and methyltransferases (for example G9a) (Ballas et. al. 2005).

Histone deacetylation by HDAC results in chromatin compaction and decrease of gene expression. Widespread alterations in histone acetylation patterns have been identified during tumorigenesis. Interestingly, HDAC inhibitors such as valproic acid (VPA) have been demonstrated to possess anti-tumor activity. It has been reported that REST is expressed not only in non-neuronal cells but also in various types of tumors. Previous studies have confirmed that REST is expressed also in HeLa cell line, a well established model of cervical adenocarcinoma – the second most common cancer in women worldwide.

The aim of this study was to assess whether pharmacologic manipulation of REST and its corepressors would promote changes in REST expression, levels of histone acetylation, since REST causes chromatin compaction through HDAC recruitment or induce apoptosis since REST overexpression reduces apoptosis in HeLa cells caused by anti-Fas antibody plus PD 98059 that suggests that REST has antiapoptotic effect in these cells (Baiula et. al. 2012).

HeLa cells, obtained from the American Type Culture Collection (Rockville, MD, USA), were transfected with 10μg/dish of REST expressing plasmid pCMV-Tag2B+REST using PEI Transfection Reagent, cells were cultured in Dulbecco's modified Eagle's medium containing 10% FCS. Not transfected cells were used as a control. After 24h of transfection cells were treated with 1.5 mmol/L VPA (Sigma). Nuclear proteins were extracted 24h after VPA treatment using NE-PER Extraction Reagent (Pierce, Rockford, IL, USA) and 50 μg of each sample were subjected to PAGE. Western Blot was performed with following antibodies: anti-REST (1:2000, Millipore) and anti-AcH3 (1:2000, Millipore).

Analysis of Western Blot on REST protein levels showed that HeLa cells treatment with VPA does not significantly affect REST expression in both cases: native REST expression and with REST transfected plasmid. However, REST protein level in transfected cells increased on 41% that suggests that transfection is efficient. Levels of acetylated form of H3 histone was not changed with REST overexpression (using REST-expressing plasmid), while VPA treatment increased on 200%. Interestingly, in HeLa cells both transfected with plasmid and treated with VPA levels of Ac-H3 were maintained high as in case with VPA treatment only. These data suggest that Ac-H3 levels increased by VPA are not influenced by REST levels.

Baiula, M., Carbonari, G., Dattoli, S., Calienni, M., Bedini, A., Spampinato, S. (2012) REST is up-regulated by epidermal growth factor in HeLa cells and inhibits apoptosis by influencing histone H3 acetylation. Biochimica et Biophysica Acta 1823 1252-1263.

Ballas, N., Grunseich, C., Lu, D.D., Speh, J.C., and Mandel, G. (2005). REST and its corepressors mediate plasticity of neuronal profiles were processed to identify statistically significant transi- gene chromatin throughout neurogenesis. Cell 121, 645–657.