REST IS DOWN-REGULATED IN NEUROBLASTOMA CELLS EXPOSED TO PMA AND RESTORED BY A PROTEASE INHIBITOR SHOWING AN INVERSE RELATIONSHIP WITH THE ACQUISITION OF NEURAL PHENOTYPES

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In this study we investigated RE-1 silencing transcription factor (REST) [1] expression pattern in SH-SY5Y neuroblastoma cells exposed to the differentiating agent phorbol 12-myristate 13-acetate (PMA; 16nM). REST expression is modified, in a time-dependent manner; this transcription factor is up-regulated in cells exposed for three days to PMA whereas, at later stages, it is down-regulated. In order to relate the decreased REST expression with a progressive neurite extension, we investigated any possible involvement of the ubiquitin–proteasome system (UPS), a multi-enzymatic pathway which degrades polyubiquinated soluble cytoplasmic proteins [2]. In SH-SY5Y cells exposed to PMA 16 nM for 4 days, the concomitant exposure to the proteasome inhibitor MG132 (1μM) restores REST nuclear protein levels down-regulated by the phorbol ester, as ascertained by *western blotting* analysis. The blockade of REST degradation is accompained by a significant reduction of neuronal differentiation markers and arrest of neurite extension. In SH-SY5Y exposed to PMA and MG 132, we observed an inverse pattern of expression of synapsin I and β- tubulin III, two neuronal differentiation markers regulated by REST. Their cytoplasmic levels are reduced when compared to cells exposed to PMA alone, as a consequence of the increase of REST expression by proteasome inhibitor. The majority of proteasome substrates identified to date are marked for degradation by polyubiquitinylation; however, exceptions to this principle, are well documented [3]. Interestingly, REST degradation seems to be completely ubiquitin-independent as we observed using two different experimental approaches. Affinity chromatography for polyubiquitinated proteins was not able to identify any REST protein; these data were confirmed by immunoprecipitation experiments performed with a specific antibody against REST. Thus, we propose that REST is regulated by PKC activators in a time-dependent manner: it is elevated during early steps of neural induction by PMA and could contribute to down-regulate genes not yet required by the differentiation program while it declines later for the acquisition of neural phenotypes, concomitantly with a progressive neurite extension. This later decline is regulated by the proteasome system activation in an ubiquitin-indipendent way and adds more evidences to the hypothesis that REST down-regulation contributes to differentiation and arrest of proliferation of neuroblastoma cells.

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2.Pickart C.M., Cohen R.E. (2004) Nat.Rev.Mol.Cell.Biol. 5: 177-187.
3.Hoyt MA, Coffino P. (2004) Cell Mol Life Sci. 61(13):1596-600.