EFFECTS OF A NOVEL HDAC INHIBITOR FR235222 IN THE HUMAN PROMONOCYTIC CELL LINE U937: ROLE OF ANNEXIN A1

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Annexin A1 (ANXA1) is the first characterized member of the annexin superfamily of proteins, so called since their main property is to bind (i.e. to annex) to cellular membranes in a Ca\textsuperscript{2+}-dependent manner. ANXA1 is commonly dysregulated in several cancers and the frequent down-regulation has also suggested a possible homeostatic or tumor suppressor role (1, 2). Histone deacetylases (HDACs) are enzymes involved in the remodelling of chromatin and have a key role in the epigenetic regulation of gene expression. In recent years, inhibition of HDACs has emerged as a potential strategy to reverse aberrant epigenetic changes associated with cancer. FR235222 is a novel immunosuppressant with potent inhibitory effects on mammalian HDAC activity. As the molecular basis of HDAC antitumor selectivity remains largely unknown, the aim of this study was to investigate the effects of the novel HDAC inhibitor FR235222 in the human promonocytic cell line U937 and to investigate the involvement of ANXA1 in this mechanism. We first investigated the effect of FR235222 on histone acetylation in U937 by Western blotting. Time-course experiments showed that FR235222 (50 nM) increased acetylation of histone H4 at 24 h (n=3, P<0.001). We next investigated the effect of FR235222 (50 nM) on growth and survival of U937 cells. FR235222 was able to reduce the growth of U937 cells. In order to investigate the effect of the FR235222 on mitotic arrest, we analyzed cell cycle on U937 cells. Time course analysis of cell-cycle progression after FR235222 showed an increase in the percentage (90%) of G0/G1 phase cells at 24 h. Growth arrest is associated with up-regulation of p21. The role of ANXA1 in the FR235222 effect was then investigated. Time-course experiments showed that FR235222 (50 nM) stimulated the cytosolic expression of ANXA1 in a time-dependent fashion with a peak at 24 h (n=3, P<0.001). In addition, ANXA1 mRNA was significantly increased in the presence of FR235222, suggesting a transcriptional regulation. These results indicate that FR235222 induce growth arrest of U937 cells and G0/G1-phase block at 24 h. Moreover the FR235222 regulate the cytosolic expression of ANXA1 at the transcriptional level. These findings suggest that ANXA1 gene expression could be governed by epigenetic changes and may explain the protein down-regulation in several cancers.