



## **“Effects of HDACs in proliferation and apoptosis of human leukaemia cell lines: Role of Annexin A1 and its membrane localization”**

Walter d'Acunto, PhD  
Dipartimento Scienze Farmaceutiche  
Università degli Studi di Salerno  
Italia

### Abstract

Annexin A1 (ANXA1) belongs to a family of ubiquitous phospholipid and calcium binding proteins. These proteins were initially described as potent inhibitors of phospholipase A2. Later, ANXA1 was implicated in several processes including involvement in the regulation of membrane trafficking and exocytosis, mediation of cytoskeleton-membrane interactions, mitogenic signal transduction, cell proliferation and differentiation as well as apoptosis (1). ANXA1 is commonly dysregulated in several cancers and the frequent down-regulation has also suggested a possible homeostatic or tumor suppressor role (2). Annexin A1 is highly susceptible to proteolytic cleavage within the N-terminal domain where cleavage sites for proteases have been identified. ANXA1 mainly exists in the cytosol, but it is also detected in the membrane or the nucleus. Recent reports suggest that subcellular localization of ANXA1 can be redistributed by treatment with specific stimuli, inducing phosphorylation, cleavage and translocation of the protein to the membrane (3). The phosphorylation on serine 27 of ANXA1 is essential for the exportation of the protein to the cell surface (4). Histone deacetylases (HDACs) represent a family of enzymes that cooperate with histone acetyltransferases (HATs) to modulate chromatin structure and transcriptional activity via changes in the acetylation status of nucleosomal histones. In addition to chromatin remodelling, the HDAC enzyme complex has been linked with several important regulatory pathways for growth differentiation and apoptosis. We have studied the mechanisms of the anti-leukemic effects of FR235222, a novel immunosuppressant with potent inhibitory effects on mammalian HDAC activity. We found that FR235222 (50 nM) caused accumulation of acetylated histone H4, inhibition of cell proliferation and G1 cycle arrest accompanied by increase of p21 and decrease of cyclin E levels in human promyelocytic leukaemia U937 cells. The role of ANXA1 in FR235222 effect was then investigated. The cytosolic expression of ANXA1, but not the membrane localization, was stimulated after 24h treatment with the drug (n=3; p<0.001). ANXA1 mRNA was significantly increased in the presence of FR235222, suggesting a transcriptional regulation. Furthermore FR235222 (0.5  $\mu$ M) was also able to

induce apoptosis in U937 cells, associated to the activation of caspase-3 as well as to an increased expression of the full-length (37kDa) ANXA1 and to the appearance of the 34 kDa N-terminal cleavage product in both cytosol and membrane. The transport of several proteins requires the function of an ATP-binding cassette (ABC) transporter by a non-classical secretion pathway. Previous studies have shown that ABCA1 is co-localized with ANXA1 surface membrane (5). On the basis of these evidences we decided to study the effect of FR235222 in presence of pan-ABC (Glyburide) and ABCA1 (Sulphobromophtalein) transporter inhibitors. We observed a reduction of ANXA1 membrane expression levels induced by FR235222 in presence of both the inhibitors that was more pronounced with sulphobromophtalein treatment. Moreover, FR235222-mediated apoptosis was partially reduced during the co-treatment with both inhibitors. Our studies suggest that the pro-apoptotic effect of FR235222 is partially mediated by ANXA1. The increase of cytosolic ANXA1 expression appears not to be sufficient to trigger the apoptotic event, rather the death signal induced by FR235222 requires the translocation of ANXA1 to the membrane. Further investigations will address the precise role of membrane ANXA1 in apoptosis.