EXPRESSION OF ANNEXIN A1 IN MOUSE MYOBLAST DIFFERENTIATION CELL LINE

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Annexin A1 (ANXA1) is a member of the calcium-dependent phospholipid binding protein family. ANXA1 has been involved in several biological processes such as ant-inflammation, cell signalling, apoptosis and differentiation. The expression of ANXA1 changes during cell differentiation and embryonic development. Correlation of ANXA1 up-regulation with expression of a marker for differentiation was observed in embryonic skin development, A459 epithelial cell line, and erythroid differentiation (1). The aim of this work was to investigate the role of ANXA1 in the differentiation of murine C2C12 myoblast cell line. We first investigated whether expression of ANXA1 is regulated during C2C12 myoblast differentiation. Cytosolic ANXA1 expression was detected by Western blotting analysis and fluorescence microscopy. Time-course experiments showed that the highest levels of ANXA1 expression was detected at 3, 5 and 7 days of differentiation ($n=3$, $P<0.001$). As annexin phosphorylation is required for protein translocation to the membrane (2), ANXA1 and p-Ser27/ANXA1 expression on the cell membrane was investigated by Western blotting and fluorescence microscopy. The highest levels of membrane ANXA1 expression was observed at 3, 5 and 7 days of differentiation while the highest levels of membrane p-Ser27/ANXA1 expression were observed at 3 days of differentiation with very low levels at 7 days. These results were also confirmed by fluorescence microscopy. In order to investigate the possibility that p-Ser27/ANXA1 could be secreted in differentiated cells, we investigated the presence of p-Ser27/ANXA1 in supernatant of cells during myoblast differentiation by immunoprecipitation and Western blotting analysis. P-Ser27/ANXA1 presence in the supernatant was observed at 4 days of differentiation. Finally, to determine ANXA1 implication in myogenesis, C2C12 mouse myoblasts were exposed to the peptide Ac2-26 (ANXA1 N-terminal portion mimetic peptide) with differentiation medium, using MHC expression as a differentiation marker. MHC expression was detected by Western blotting analysis. Treatment of C2C12 with peptide Ac2-26 resulted in a significant increase of MHC expression in cells at 4 days of differentiation, compared with cells treated only with differentiation medium. ($n=3$, $P<0.001$). Our results indicate, for the first time, that ANXA1 plays some critical, but as yet undefined, role in myoblast differentiation.

1. Huo X.F. and Zhang J.W. (2005) Biochem Biophys Res Commun. 331: 1346-1352.
2. Kim Y.S., Ko J., Kim I.S., Kim Y. and Na D.S. (2003) Eur J Biochem. 270: 4089-4094.