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Molecular imaging: a FLIM-based FRET assay to identify novel protein-protein interactions between Cx43 and Cdo during myoblast differentiation

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Previous studies of our group have shown that connexin43 (Cx43), besides forming gap junction channels, serves as a signaling center by binding to proteins of the actin cytoskeleton during skeletal myoblast differentiation. There is also evidence that Cdo (CAM-related/down regulated by oncogenes), a member of the Ig/FNIII family of cell surface receptors, exerts its promyogenic effects acting as a component of multiprotein complexes containing cytoskeletal components. Within these signaling platform, the interaction of Cdo and the cell-cell adhesion molecule N-cadherin is essential for the activation p38MAPK and the induction of myogenesis. Of interest, cadherins are also closely associated with Cx43 at sites of cell-to-cell contacts to ensure gap junction intercellular trafficking and functional integration. On these bases, in the present study we searched for a possible interaction of Cx43 and Cdo in differentiating C2C12 cells, using either optical methods such as confocal microscopy, fluorescence spectral analysis, FLIM-FRET (Fluorescence Lifetime Imaging -Fluorescence Resonance Energy Transfer) techniques and co-immunoprecipitation experiments.

It was found that Cx43 and Cdo expression significantly correlated with each other during myoblast differentiation; both molecules reached the highest expression levels at 24 h and gradually disappeared with the ongoing of differentiation. Interestingly, stimulation with S1P, a bioactive lipid and a potent stimulator of myogenesis greatly potentiated the expression of Cx43 and Cdo in differentiating C2C12 cells. Moreover, Cx43 and Cdo co-localized at the plasma membrane and generated, especially in the cells stimulated with S1P, a strong FRET signal, as judged by either spectral and FLIM analyses, consistent with a physical interaction between the two proteins. These results matched the immunoprecipitation data. In conclusion, these results provide the first evidence for a direct interaction between Cx43 and Cdo in differentiating myoblasts and raise the intriguing hypothesis that this interaction may be a key step in the gap junction independent Cx43-mediated signaling.

Key words
C2C12 cells, Connexin 43, Cdo, myogenesis, FRET