

Detection of Cellular PS-exposure by Lactadherin.

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Recognition and clearance of apoptotic cells is a vital process for multicellular organisms. An early feature of apoptosis is the redistribution of the membrane lipid phosphatidylserine (PS) to the extracellular surface of the plasma membrane. PS has been recognized as an eat-me signal for both professional and non-professional phagocytes either directly through the PS receptor or mediated through opsonins. One such opsonin is the protein lactadherin (MFG-E8). Animal studies have shown that lactadherin functions as an effective opsonin between the PS on the apoptotic cells and the $\alpha_v\beta_3$ or $\alpha_v\beta_3$ integrin receptors (RGD mediated) on the phagocyte. We have utilized the inherent ability of lactadherin to recognize PS in studies validating its potential as an *in vitro* imaging agent of apoptosis. This has been done utilizing conjugation products of purified bovine lactadherin and either fluorescein or hydrazinonicotinamide (HYNIC) and ^{99m}Tc. Biological activity of the compounds and their selectivity for PS was estimated on apoptotic HL60 and K562 cell lines by means of flow cytometry and confocal microscopy for fluorescein and conventional binding assays for ^{99m}Tc. Eventual binding through the integrin-binding EGF-like domains of lactadherin was ruled out through inhibition with RGD peptides. All experiments indicate that the lactadherin conjugates are indeed stable and biologically active with a specific affinity for PS containing membranes. Lactadherin thus shows promise as a new tool for Ca²⁺ independent PS imaging, and might allow experimental setups where the benchmark PS recognizing protein Annexin V cannot be utilized.