Identification and Characterization of the Acidic pH Binding Sites for Growth Regulatory Ligands of Low Density Lipoprotein Receptor-related protein-1

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摘要.

The type V TGF-β receptor (TβR-V) plays an important role in growth inhibition by IGFBP-3 and TGF-β in responsive cells. Unexpectedly, TβR-V was recently found to be identical to the LRP-1/α2M receptor; this has disclosed previously unreported growth regulatory functions of LRP-1. Here we demonstrate that, in addition to expressing LRP-1, all cells examined exhibit low affinity but high density acidic pH binding sites for LRP-1 growth regulatory ligands (TGF-β1, IGFBP-3, and α2M*). These sites, like LRP-1, are sensitive to receptor-associated protein and calcium depletion but, unlike LRP-1, are also sensitive to chondroitin sulfate and heparin and capable of directly binding ligands, which do not bind to LRP-1. Annexin VI has been identified as a major membrane-associated protein capable of directly binding α2M* at acidic pH. This is evidenced by: 1) structural and Western blot analyses of the protein purified from bovine liver plasma membranes by α2M* affinity column chromatography at acidic pH, and 2) dot blot analysis of the interaction of annexin VI and 125I-α2M*. Cell surface annexin VI is involved in 125I-TGF-β1 and 125I-α2M* binding to the acidic pH binding sites and 125I-α2M* binding to LRP-1 at neutral pH as demonstrated by the sensitivity of cells to pretreatment with anti-annexin VI IgG. Cell surface annexin VI is also capable of mediating internalization and degradation of cell surface-bound 125I-TGF-β1 and 125I-α2M* at pH 6 and of forming ternary complexes with 125I-α2M* and LRP-1 at neutral pH as demonstrated by co-immunoprecipitation. Trifluoperazine and fluphenazine, which inhibit ligand binding to the acidic pH binding sites, block degradation after internalization of cell surface-bound 125I-TGF-β1 or 125I-α2M*. These results suggest that cell surface annexin VI may function as an acidic pH binding site or receptor and may also function as a co-receptor with LRP-1 at neutral pH.