LDL receptor-related protein: Molecular analysis and identification of new ligands
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Summary
Chapter 7


The low density lipoprotein receptor-related protein (LRP) is a large multifunctional endocytic cell-surface receptor that binds and internalizes an impressive number of structurally-unrelated classes of ligands. This broad range of ligands suggests a role for the receptor in diverse physiological and pathophysiological processes ranging from lipoprotein metabolism, fibrinolysis, cell growth and cell migration, to thrombosis, atherosclerosis and Alzheimer's disease.

In Chapter 1 of this thesis, an overview is presented of the biology of LRP. The gene structure, biosynthesis, as well as the tissue distribution and regulation of cellular expression of LRP, are described. The ligands and their relation to (patho)physiological functions of LRP are outlined, together with the structural determinants on both LRP and ligands responsible for ligand binding. Emphasis is given to the molecular mechanisms that enable LRP to interact specifically with such a multitude of different ligands.

In Chapter 2, we demonstrate that coagulation factor VIII is a ligand of LRP. In a system, consisting of purified components, it was shown that factor VIII binds to LRP in a reversible and dose-dependent manner. Furthermore, in the presence of the factor VIII carrier protein vWF or the LRP antagonist RAP, this binding is efficiently inhibited, emphasizing the specificity of the interaction. Both anti-LRP antibodies and RAP interfered with cellular degradation of factor VIII. In addition, degradation of factor VIII was completely inhibited by vWF. Because vWF binding to factor VIII is mediated by the factor VIII light chain, we specifically studied LRP binding to this subunit. These experiments revealed that factor VIII light chain indeed binds to LRP. Furthermore, experiments using recombinant factor VIII C2 domain showed that this part of the factor VIII light chain contributes to the interaction with LRP. Collectively, this study demonstrates that LRP is able to bind factor VIII at the cell surface in vitro and to mediate its transport to the intracellular degradation pathway. The regulatory role of factor VIII-vWF complex formation in LRP binding may explain the beneficial effect of vWF on the in vivo survival of factor VIII.

In Chapter 3, soluble recombinant receptor-fragments were used, representing the four putative ligand-binding domains of LRP, generally referred to as clusters I, II, III, and IV, to map the binding sites of a set of structurally and functionally distinct ligands. By this systematic examination, more insight into the molecular elements that contribute to the remarkable ligand binding capacity of LRP was obtained. Although there are small differences concerning the kinetics of the interactions, it was demonstrated that clusters II and IV are highly similar in their ligand-binding properties, revealing a major functional duplication in the receptor. In addition, it was shown in this study that a single RAP molecule can simultaneously bind to clusters II and IV with similar affinities. This observation is in accordance with a model for inhibition of ligand binding to LRP by RAP, in which one molecule of RAP can induce a conformational change in the receptor by interacting simultaneously with multiple receptor domains. This conformational change would then render the receptor incapable of ligand binding.

In Chapter 4, the interaction between LRP and either coagulation factor IX or its active derivative factor IXa was studied. Although factor IX was unable to bind to LRP, factor XIa mediated conversion of factor IX into factor IXa resulted in reversible dose- and calcium-dependent binding to LRP. This observation suggests that activation of factor IX results in exposure of a binding site for LRP. Since active-site blocking of
factor IXa did not affect binding to LRP, this exposed binding site is thought to reside outside the exposed active site. LRP binding was efficiently inhibited in the presence of heparin or antibodies against factor IX or LRP. In addition, degradation of factor IXa was studied using LRP-expressing, LRP-deficient, and proteoglycan-deficient cells. These experiments indicate that catabolism of factor IXa involves both LRP and cell-surface proteoglycans.

In Chapter 5, the possible implications of the identification of coagulation factor VIII and factor IXa as novel ligands of LRP are reviewed. These findings, together with the observation of others that LRP is involved in down-regulation of Tissue Factor expression at the surface of monocytes and fibroblasts, suggest a potential contribution of LRP to the regulation of the coagulation cascade. Specifically, LRP might serve a so far unrecognized role in modulation of the coagulation cascade in both initiating (Tissue Factor) and propagating (factor VIII, factor IXa) stages of the coagulation process.

In Chapter 6, a soluble form of LRP (sLRP) was subject of investigation. Using an ELISA, the serum levels of sLRP were measured in 50 normal individuals and 61 Gaucher patients. The mean soluble LRP level was increased 2.7 fold in sera of these patients as compared to the mean sLRP serum-level measured in the normal individuals. This is the first study reporting increased serum-levels of sLRP in an inborn error of metabolism. When Gaucher patients were treated with either enzyme supplementation therapy or the novel oral substrate-reduction therapy, this resulted in normalization of sLRP serum-levels. Therefore, measurement of serum sLRP levels is of potential interest in connection with monitoring efficacy of therapeutic intervention.

Finally, in Chapter 7, a general discussion is presented of the work reported in this thesis in the context of the most recent developments. The discussion predominantly concentrates on the current knowledge on the structure and function relations of LRP and molecular elements that contribute to the impressive ligand binding capacity of LRP. Special attention is paid to the novel function of LRP as a transducer of extracellular signals, the correlation of LRP with Alzheimer’s disease, sLRP, and the most recent developments concerning ligand binding to LRP.