FEBS 29182 FEBS Letters 579 (2005) 773–777

The endocytic receptor megalin binds the iron transporting neutrophil-gelatinase-associated lipocalin with high affinity and mediates its cellular uptake

Vibeke Hvidberg[a], Christian Jacobsen[a], Roland K. Strong[b], Jack B. Cowland[c], Søren K. Moestrup[a], Niels Borregaard[c,*]

[a] *Institute of Medical Biochemistry, University of Aarhus, Denmark*
[b] *Division of Basic Sciences, Fred Hutchinson Cancer Research Center, USA*
[c] *The Granulocyte Research Laboratory, Department of Hematology, Rigshospitalet 4042, 9 Blegdamsvej, DK-2100 Copenhagen Ø, Denmark*

Received 17 November 2004; revised 1 December 2004; accepted 1 December 2004

Available online 24 December 2004

Edited by Lukas Huber

Abstract Neutrophil-gelatinase-associated lipocalin (NGAL) is a prominent protein of specific granules of human neutrophils also synthesized by epithelial cells during inflammation. NGAL binds bacterial siderophores preventing bacteria from retrieving iron from this source. Also, NGAL may be important in delivering iron to cells during formation of the tubular epithelial cells of the primordial kidney. No cellular receptor for NGAL has been described.

We show here that megalin, a member of the low-density lipoprotein receptor family expressed in polarized epithelia, binds NGAL with high affinity, as shown by surface plasmon resonance analysis. Furthermore, a rat yolk sac cell line known to express high levels of megalin, endocytosed NGAL by a mechanism completely blocked by an antibody against megalin.
© 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.

Keywords: NGAL; Lipocalin; Megalin; Siderophore; Iron

1. Introduction

Lipocalins constitute a family of more than 30 proteins, found in species as separate as insects and man [1,2]. Lipocalins share a common structural fold, often in the absence of significant sequence homology, that forms a ligand-binding site (calyx) that is typically lined with hydrophobic residues [3,4]. Lipocalins function in general as transport proteins, e.g., odorant-binding protein, bilin-binding protein, and retinol-binding protein, but the physiological ligands have not been identified for all lipocalins. Lipocalins are extracellular proteins but some are involved in transport of substances that must be delivered intracellularly to exert a function, e.g., retinol. Very little is known about the receptors that mediate cellular uptake of lipocalins and their ligands. It is not known whether the ligands are presented by the lipocalins to ligand receptors on cells or whether the lipocalins are taken up by receptors and then deliver their cargo intracellularly [5]. It is assumed that most lipocalins are taken up by membrane receptors but only two have been identified so far. One is a novel 51 kDa protein which binds tear-lipocalin (lipocalin-1 (Lcn-1)) and consequently is known as Lcn-1-Interacting Membrane Protein [6,7]. The other receptor is megalin [8], a multi-ligand endocytosis receptor that is expressed on a variety of epithelia, primarily such that have a high absorptive capacity such as tubular epithelial cells of kidneys, ileum, choroid plexus, and yolk sac [9]. Megalin belongs to the low density lipoprotein receptor family [8]. Megalin has been shown to bind the mouse lipocalins retinol-binding protein, α_1-microglobulin, mouse major urinary protein, and odorant-binding protein [5,10].

We discovered a major human lipocalin, neutrophil-gelatinase-associated lipocalin (NGAL) (also known as lipocalin-2 (lcn-2) or siderocalin), and have characterized its tissue expression [11]. NGAL is constitutively expressed in human neutrophils but is also induced in epithelial cells when these are engaged in inflammation [12–14]. This tissue expression indicates that NGAL is involved in innate immunity. This notion was supported when siderophores were identified as NGAL ligands [15] and followed by the recent demonstration of decreased survival of mice with a targeted disruption of the NGAL (lcn-2) after intraperitoneal challenge with *Escherichia coli* [16]. Siderophores are extremely strong iron chelators secreted by microorganisms when iron is limiting [17]. Siderophores can extract iron out of iron-binding proteins such as transferrin and lactoferrin for subsequent uptake by specific siderophore receptors on the microorganisms. In this way, microorganisms can secure a supply of this essential nutrient at the expense of their host [17]. It has been shown that apo-NGAL is able to prevent siderophore producing *E. coli* from dividing and that this restrain on bacterial growth is alleviated by supplying iron in excess of the iron-siderophore binding capacity of NGAL [15]. However, such antimicrobial activity can be expected to be temporary because several microorganisms secrete proteases that may degrade NGAL even though NGAL is known to be a very protease resistant molecule [18]. NGAL would therefore be expected to have a more sustained antimicrobial effect, if NGAL is taken up by host cells.

*Corresponding author. Fax: +45 3545 4295.
E-mail address: borregaard@rh.dk (N. Borregaard).

Abbreviations: BN, Brown Norway; Lcn-1, lipocalin-1; Lcn-2, lipocalin-2; LRP, LDL-receptor related protein; NGAL, neutrophil-gelatinase-associated lipocalin; RAP, receptor associated protein; SPR, surface plasmon resonance

0014-5793/$30.00 © 2004 Federation of European Biochemical Societies. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.febslet.2004.12.031

This has been further indicated by the recent observation that the mouse ortholog of NGAL, 24p3, is expressed in the fetal kidney and delivers iron to cells at the stage where differentiation of the budding kidney epithelia takes place during organogenesis of the kidneys [19,20]. The rapid clearance of NGAL from the circulation indicates a mechanism of cellular uptake [21]. In addition, NGAL has been inferred as a factor inducing apoptosis through a receptor mediated mechanism in IL-3 dependent cells such as bone marrow cells in the mouse [22], but this has not been confirmed [23].

Since none has yet been identified, we decided to test whether megalin could also function as a receptor for NGAL.

2. Materials and methods

Recombinant human NGAL was generated either as apo-NGAL, i.e., without bacterial siderophore, in Sf9 insect cells using the Baculovirus technique as previously described [24], or as NGAL with *E. coli* siderophore as detailed in [15,25], with the modification that 50 ml overnight culture of *E. coli* was added to 500 ml LB medium and incubated for 90 min. Then, 0.1 mM IPTG and 0.1 mg/ml desferoxamine (Desferal, Roche) were added to induce bacterial growth (IPTG) and secure iron deprivation and enterobactin production by the bacteria. After 3 h, 0.1 mg/ml $FeCl_3$ was added and the bacteria incubated for another 90 min. The bacteria were then pelleted by centrifugation and formed a bright red pellet. The bacteria were lysed and rhNGAL was isolated as described [25]. The isolated NGAL was scanned as described from 250 to 800 nm, and showed the characteristic peaks at 330 and 500 nm indicative of enterobactin–Fe^{3+} complex in the lipocalin pocket [15]. These were absent in the apo-NGAL from Sf9 cells (Fig. 1). It was ascertained that desferoxamine did not bind to apo-NGAL by incubating apo-NGAL with desferoxamine and $FeCl_3$.

Mouse monoclonal anti-NGAL antibody was used for Western blotting [26]. NGAL was quantitated as described by Kjeldsen et al. [26].

Megalin was purified by receptor-associated protein (RAP) affinity chromatography from human kidney cortex according to standard procedures [27]. Human LDL-receptor related protein (LRP) was purified by α_2 macroglobulin-affinity chromatography as described [28]. Purified sheep polyclonal antibodies against rat megalin, previously described [29], were used to block uptake of NGAL by megalin. Purified sheep non-immune IgG served as negative control.

3. Surface plasmon resonance analysis

The binding to megalin and LRP was studied by surface plasmon resonance (SPR) analysis on a Biacore 2000 instrument (Biacore, Sweden). The procedure was as follows: Biacore sensor chips type CM5 were activated with a 1:1 mixture of 0.2 M *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide and 0.05 M *N*-hydroxysuccimide in water according to the manufacturer's instructions. Megalin and LRP were immobilized at a concentration of 10 μg/ml in 10 mM sodium acetate, pH 4.5, and the remaining binding sites were blocked with 1 M ethanolamine, pH 8.5. A control flow cell was made by performing the activation and blocking procedures only. The resulting receptor densities were 25–30 fmol receptor/mm^2. Samples were dissolved in 10 mM HEPES, 150 mM NaCl, 1.5 mM $CaCl_2$, 1.0 mM EGTA, and 0.005% Tween 20, pH 7.4, or in 10 mM HEPES, 150 mM NaCl, 20 mM EGTA, and 0.005% Tween 20, pH 7.4. Sample and running buffer were identical. Regeneration of the sensor chip after each analysis cycle was performed with 1.6 M glycine–HCl buffer, pH 3.0. The Biacore response is expressed in relative response units (RU), i.e., the difference in response between protein and control flow channel (an activated but uncoupled flow cell). Kinetic parameters were determined by BIAevaluation 4.1 software using a Langmuir 1:1 binding model and simultaneous fitting of all curves in the concentration range considered (global fitting).

4. Cellular uptake of NGAL

Megalin-expressing Brown Norway (BN) rat yolk sac epithelial cells transformed with mouse sarcoma virus (BN cells) [30] were grown on two-chamber Permanox slides (Nunc, Roskilde, Denmark) and washed in PBS, pH 7.4. Cells were then incubated for 1 h at 37 °C in HyQ-CCM5 serum-free medium (Hyclone, Logan, Utah) containing 1% BSA (w/v) and ≈60 μg/ml rhNGAL labeled with Alexa-488 (Molecular Probes, Leiden, The Netherlands). To some cells was also added sheep polyclonal anti-rat megalin IgG antibody (200 μg/ml) or sheep non-immune IgG antibody (200 μg/ml). After incubation, cells were washed in PBS, pH 7.4, and fixed in 4% formaldehyde for 1 h at 4 °C. Subsequently, cells were washed in PBS, pH 7.4, containing 0.05% Triton X-100 and incubated with rabbit anti-rat cubilin antibody [31] (10 μg/ml) in this buffer for 1 h at room temperature in order to visualize the megalin-expressing structures of the cells. After washing in PBS, 0.05% Triton X-100, cells were incubated for 1 h at room temperature with Alexa-594-conjugated secondary anti-rabbit IgG (Molecular Probes) diluted 1:200 in the same buffer. Slides were washed in PBS, pH 7.4, mounted in Dako fluorescent mounting medium (DakoCytomation, Glostrup, Denmark) and analyzed

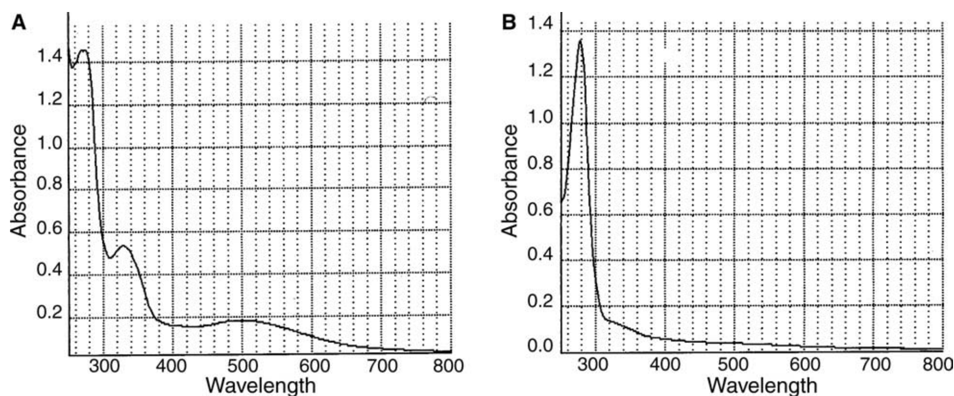

Fig. 1. Absorption spectrum of NGAL. NGAL (1.2 mg/ml) with siderophore (A) and without siderophore (B) in PBS was scanned against PBS.

using a Zeiss LSM-510 confocal microscope (Zeiss, Jena, Germany).

5. Results

To test the hypothesis that NGAL might bind to megalin, studies were conducted using SPR technique with matrix bound megalin. Fig. 2 shows that binding ($K_d = \sim 60$ nM) of apo-NGAL to megalin occurs with high affinity. Similar affinity was measured with siderophore-bound NGAL (not shown). The NGAL binding was prevented by EDTA, indicating that the LDL-receptor type-A repeats of megalin are involved in the binding of NGAL. Since this motif is also found in the LRP [32], we tested the binding of NGAL to LRP. However, only a very weak signal was recorded (not shown) indicating that this receptor has no major role in NGAL clearance.

The role of megalin in cellular uptake of NGAL was further investigated in a rat yolk sac cell line that is known to express high amounts of megalin [30]. Fig. 3 shows that endocytosis of fluorescently labeled NGAL by this cell line was extensive as seen by the appearance of intracellular NGAL (Alexa-488, green flourescence). A sheep polyclonal anti-megalin antibody completely prevented cellular uptake of NGAL by these cells (Fig. 3B), thus indicating that megalin is involved in mediating the cellular uptake of NGAL by the cells. Cubilin, a receptor colocalizing with megalin and recycling between coated pits and endosomes, was visualized with Alexa-594 coupled antibody (red flourescense). The different localization of NGAL and cubilin indicates that internalized NGAL is not recycled but segregated from the receptor and targeted to endosomes and lysosomes.

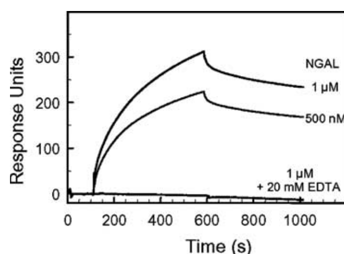

Fig. 2. Surface plasmon resonance analysis of the binding of NGAL to purified megalin. Binding of 500 nM and 1 μM NGAL to megalin. No binding of NGAL was seen in the presence of calcium-complexing EDTA.

6. Discussion

Neutrophil-gelatinase-associated lipocalin appears to be a protein of importance in iron metabolism both during organogenesis [19,20] and in host defense [15,16], and possibly also in tumorigenesis [33,34]. The ability of NGAL to sequester iron and thus prevent its utilization by growing microorganisms has been demonstrated in vitro [15], and NGAL/24p3 has been shown to mediate uptake of iron by the developing kidney [19]. Although not a priori apparent, it is highly likely that the ability of NGAL to prevent iron utilization by microorganisms as demonstrated in vitro is ultimately dependent on cellular uptake of iron, since NGAL might be degraded by (microbial) proteases and the siderophore-bound iron retrieved by microorganisms if NGAL is not taken up by the host epithelial cells. Certainly, the essential role of NGAL in inducing epithelial cell differentiation in the developing kidney is dependent on cellular uptake and delivery of NGAL bound iron to the epithelial cells [19]. It is similarly likely – but entirely speculative – that the expression of NGAL by a variety of epithelial tumors endows these with an iron retrieving mechanism that adds to the growth potential of the tumors [33–35], and thus that for NGAL to exert its function, be it in host defense or during cellular growth and differentiation, a cellular receptor is needed which mediates uptake of NGAL with high affinity. We demonstrate here that megalin acts as such a cellular receptor for NGAL. Megalin is also known to bind another iron-binding protein expressed and secreted from human neutrophils, lactoferrin [36,37]. Although the ligands for megalin are very diverse, positively charged amino acids have been shown to be critical for binding of several ligands to this receptor [32]. The p*I* of NGAL of 8.4 distinguishes NGAL from most other lipocalins [4]. A number of positive charges can by identified on NGAL from analysis of its crystalline structure [15]. The affinity of megalin for NGAL as determined by plasmon resonance is much higher than that observed for other lipocalins and may likely relate to the unique positive charge of NGAL among lipocalins.

The tissue expression of megalin fits well with the induction of NGAL expression during inflammation. NGAL is highly expressed by type 2 pneumocytes during inflammation [12]. These also express megalin [8]. NGAL is also highly expressed by epithelial cells of the intestines during inflammation [13]. These also express megalin [9].

It is noteworthy that we were not able to show any selectivity of megalin for siderophore NGAL versus apo-NGAL. It may be argued that in this way apoNGAL may decrease the

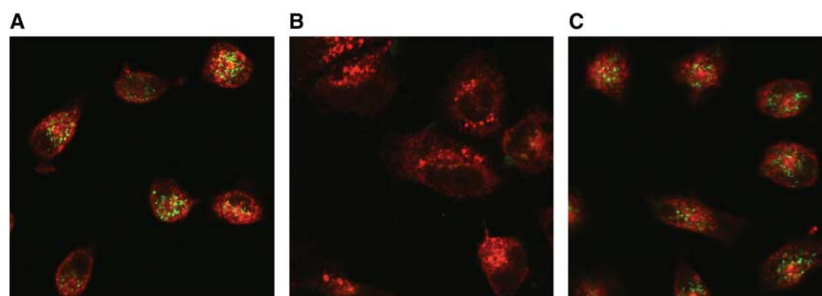

Fig. 3. Confocal fluorescence microscopy analysis of uptake of Alexa-488-labeled NGAL in BN cells. BN cells were incubated for 1 h with rhNGAL (green) in the absence (A) or in the presence (B) of anti-megalin IgG or non-immune IgG (C). The red color represents Alexa-594 staining for cubilin, a receptor colocalizing with megalin [38].

efficiency by which megalin takes up siderophore-NGAL – this would however only be a problem if the ability of megalin to bind and endocytose NGAL is a limiting factor. The studies of uptake indicate that the capacity of megalin to endocytose NGAL is very high.

Our studies do not exclude the possibility that other receptors other than megalin may be involved in cellular uptake of NGAL.

Acknowledgments: This study was supported by grants from the Danish Medical Research Council. The expert technical support of Charlotte Horn is greatly appreciated.

References

[1] Flower, D.R. (1996) The lipocalin protein family: structure and function. Biochem. J. 318, 1–14.

[2] Ganfornina, M.D., Gutierrez, G., Bastiani, M. and Sanchez, D. (2000) A phylogenetic analysis of the lipocalin protein family. Mol. Biol. Evol. 17, 114–126.

[3] Patel, R.C., Lange, D., McConathy, W.J., Patel, Y.C. and Patel, S.C. (1997) Probing the structure of the ligand binding cavity of lipocalins by fluorescence spectroscopy. Protein Eng. 10, 621–625.

[4] Akerstrom, B., Flower, D.R. and Salier, J.P. (2000) Lipocalins: unity in diversity. Biochim. Biophys. Acta 1482, 1–8.

[5] Flower, D.R. (2000) Beyond the superfamily: the lipocalin receptors. Biochim. Biophys. Acta 1482, 327–336.

[6] Wojnar, P., Lechner, M. and Redl, B. (2003) Antisense down-regulation of lipocalin-interacting membrane receptor expression inhibits cellular internalization of lipocalin-1 in human NT2 cells. J. Biol. Chem. 278, 16209–16215.

[7] Wojnar, P., Lechnar, M., Merschak, P. and Redl, B. (2001) Molecular cloning of a novel lipocalin-1 interacting human cell membrane receptor using phage display. J. Biol. Chem. 276, 20206–20212.

[8] Saito, A., Pietromonaco, S., Loo, A.K. and Farquhar, M.G. (1994) Complete cloning and sequencing of rat gp330/"megalin", a distinctive member of the low density lipoprotein receptor gene family. Proc. Natl. Acad. Sci. USA 91, 9725–9729.

[9] Moestrup, S.K. and Verroust, P.J. (2001) Megalin- and cubilin-mediated endocytosis of protein-bound vitamins, lipids, and hormones in polarized epithelia. Annu. Rev. Nutr. 21, 407–428.

[10] Leheste, J.R., Rolinski, B., Vorum, H., Hilpert, J., Nykjaer, A., Jacobsen, C., Aucouturier, P., Moskaug, J.O., Otto, A., Christensen, E.I. and Willnow, T.E. (1999) Megalin knockout mice as an animal model of low molecular weight proteinuria. Am. J. Pathol. 155, 1361–1370.

[11] Cowland, J.B. and Borregaard, N. (1997) Molecular characterization and pattern of tissue expression of the gene for neutrophil gelatinase-associated lipocalin from humans. Genomics 45, 17–23.

[12] Cowland, J.B., Sorensen, O.E., Sehested, M. and Borregaard, N. (2003) Neutrophil gelatinase-associated lipocalin Is up-regulated in human epithelial cells by IL-1beta, but not by TNF-alpha. J. Immunol. 171, 6630–6639.

[13] Nielsen, B.S., Borregaard, N., Bundgaard, J.R., Timshel, S., Sehested, M. and Kjeldsen, L. (1996) Induction of NGAL synthesis in epithelial cells of human colorectal neoplasia and inflammatory bowel disease. Gut 38, 414–420.

[14] Mallbris, L., O'Brien, K.P., Hulthen, A., Sandstedt, B., Cowland, J.B., Borregaard, N. and Stahle-Backdahl, M. (2002) Neutrophil gelatinase-associated lipocalin is a marker for dysregulated keratinocyte differentiation in human skin. Exp. Dermatol. 11, 584–591.

[15] Goetz, D.H., Holmes, M.A., Borregaard, N., Bluhm, M.E., Raymond, K.N. and Strong, R.K. (2002) The neutrophil lipocalin NGAL is a bacteriostatic agent that interferes with siderophore-mediated iron acquisition. Mol. Cell. 10, 1033–1043.

[16] Flo, T.H., Smith, K.D., Sato, S., Rodriguez, D.J., Holmes, M.A., Strong, R.K., Akira, S. and Aderem, A. (2004) Lipocalin 2 mediates an innate immune response to bacterial infection by sequestrating iron. Nature.

[17] Neilands, J.B. (1995) Siderophores: structure and function of microbial iron transport compounds. J. Biol. Chem. 270, 26723–26726.

[18] Kjeldsen, L., Johnsen, A.H., Sengeløv, H. and Borregaard, N. (1993) Isolation and primary structure of NGAL, a novel protein associated with human neutrophil gelatinase. J. Biol. Chem. 268, 10425–10432.

[19] Yang, J., Goetz, D., Li, J.Y., Wang, W., Mori, K., Setlik, D., Du, T., Erdjument-Bromage, H., Tempst, P., Strong, R. and Barasch, J. (2002) An iron delivery pathway mediated by a lipocalin. Mol. Cell. 10, 1045–1056.

[20] Yang, J., Mori, K., Li, J.Y. and Barasch, J. (2003) Iron, lipocalin, and kidney epithelia. Am. J. Physiol. Renal Physiol. 285, F9–F18.

[21] Axelsson, L., Bergenfeldt, M. and Ohlsson, K. (1995) Studies of the release and turnover of a human neutrophil lipocalin. Scand. J. Clin. Lab. Invest. 55, 577–588.

[22] Devireddy, L.R., Teodoro, J.G., Richard, F.A. and Green, M.R. (2001) Induction of apoptosis by a secreted lipocalin that is transcriptionally regulated by IL-3 deprivation. Science 293, 829–834.

[23] Kamezaki, K., Shimoda, K., Numata, A., Aoki, K., Kato, K., Takase, K., Nakajima, H., Ihara, K., Haro, T., Ishikawa, F., Imamura, R., Miyamoto, T., Nagafuji, K., Gondo, H., Hara, T. and Harada, M. (2003) The lipocalin 24p3, which is an essential molecule in IL-3 withdrawal-induced apoptosis, is not involved in the G-CSF withdrawal-induced apoptosis. Eur. J. Haematol. 71, 412–417.

[24] Strong, R.K., Bratt, T., Cowland, J.B., Borregaard, N., Wiberg, F.C. and Ewald, A.J. (1998) Expression, purification, crystallization and crystallographic characterization of dimeric and monomeric human neutrophil gelatinase associated lipocalin (NGAL). Acta Crystallogr. D. Biol. Crystallogr. 54 (Pt 1), 93–95.

[25] Bundgaard, J.R., Borregaard, N. and Kjeldsen, L. (1994) Molecular cloning and expression of a cDNA encoding NGAL: a lipocalin expressed in human neutrophils. Biochem. Biophys. Res. Commun. 202, 1468–1475.

[26] Kjeldsen, L., Koch, C., Arnljots, K. and Borregaard, N. (1996) Characterization of two ELISAs for NGAL, a newly described lipocalin in human neutrophils. J. Immunol. Meth. 198, 155–164.

[27] Moestrup, S.K., Holtet, T.L., Etzerodt, M., Thogersen, H.C., Nykjaer, A., Andreasen, P.A., Rasmussen, H.H., Sottrup-Jensen, L. and Gliemann, J. (1993) Alpha 2-macroglobulin-proteinase complexes, plasminogen activator inhibitor type-1-plasminogen activator complexes, and receptor-associated protein bind to a region of the alpha 2-macroglobulin receptor containing a cluster of eight complement-type repeats. J. Biol. Chem. 268, 13691–13696.

[28] Moestrup, S.K. and Gliemann, J. (1991) Analysis of ligand recognition by the purified alpha 2-macroglobulin receptor (low density lipoprotein receptor-related protein). Evidence that high affinity of alpha 2-macroglobulin-proteinase complex is achieved by binding to adjacent receptors. J. Biol. Chem. 266, 14011–14017.

[29] Moestrup, S.K., Birn, H., Fischer, P.B., Petersen, C.M., Verroust, P.J., Sim, R.B., Christensen, E.I. and Nexo, E. (1996) Megalin-mediated endocytosis of transcobalamin-vitamin-B12 complexes suggests a role of the receptor in vitamin-B12 homeostasis. Proc. Natl. Acad. Sci. USA 93, 8612–8617.

[30] Le Panse, S., Verroust, P. and Christensen, E.I. (1997) Internalization and recycling of glycoprotein 280 in BN/MSV yolk sac epithelial cells: a model system of relevance to receptor-mediated endocytosis in the renal proximal tubule. Exp. Nephrol. 5, 375–383.

[31] Sahali, D., Mulliez, N., Chatelet, F., Laurent-Winter, C., Citadelle, D., Roux, C., Ronco, P. and Verroust, P. (1992) Coexpression in humans by kidney and fetal envelopes of a 280 kDa-coated pit-restricted protein. Similarity with the murine target of teratogenic antibodies. Am. J. Pathol. 140, 33–44.

[32] Hussain, M.M., Strickland, D.K. and Bakillah, A. (1999) The mammalian low-density lipoprotein receptor family. Annu. Rev. Nutr. 19, 141–172.

[33] Stoesz, S.P., Friedl, A., Haag, J.D., Lindstrom, M.J., Clark, G.M. and Gould, M.N. (1998) Heterogeneous expression of the lipocalin NGAL in primary breast cancers. Int. J. Cancer 79, 565–572.

[34] Stoesz, S.P. and Gould, M.N. (1995) Overexpression of neu-related lipocalin (NRL) in neu-initiated but not ras or chemically initiated rat mammary carcinomas. Oncogene 11, 2233–2241.

[35] Furutani, M., Arii, S., Mizumoto, M., Kato, M. and Imamura, M. (1998) Identification of a neutrophil gelatinase-associated lipocalin mRNA in human pancreatic cancers using a modified signal sequence trap method. Cancer Lett. 122, 209–214.

[36] Willnow, T.E., Goldstein, J.L., Orth, K., Brown, M.S. and Herz, J. (1992) Low density lipoprotein receptor-related protein and gp330 bind similar ligands, including plasminogen activator-inhibitor complexes and lactoferrin, an inhibitor of chylomicron remnant clearance. J. Biol. Chem. 267, 26172–26180.

[37] Meilinger, M., Haumer, M., Szakmary, K.A., Steinbock, F., Scheiber, B., Goldenberg, H. and Huettinger, M. (1995) Removal of lactoferrin from plasma is mediated by binding to low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor and transport to endosomes. FEBS Lett. 360, 70–74.

[38] Moestrup, S.K., Kozyraki, R., Kristiansen, M., Kaysen, J.H., Rasmussen, H.H., Brault, D., Pontillon, F., Goda, F.O., Christensen, E.I., Hammond, T.G. and Verroust, P.J. (1998) The intrinsic factor-vitamin B12 receptor and target of teratogenic antibodies is a megalin-binding peripheral membrane protein with homology to developmental proteins. J. Biol. Chem. 273, 5235–5242.