Chapter 13
The Endocytic Collagen Receptor, uPARAP/Endo180, in Cancer Invasion and Tissue Remodeling

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Abstract uPARAP/Endo180 is a constitutively recycling endocytosis receptor of 180 kDa. It is a type-1 membrane protein and includes an N-terminal Cys-rich domain followed by a fibronectin type-II domain, eight C-type lectin-like domains, a transmembrane segment, and a small cytoplasmic domain. The receptor binds and internalizes collagen, which is then directed to lysosomal degradation. The internalization efficiency increases when the collagen is in a gelatin-like state and, in line with this notion, the uptake of defined ¼ and ¾ collagen fragments is more efficient than the internalization of intact collagen. Thus, uPARAP/Endo180 most likely has a preferential role in the clearance of precleaved collagen, occurring after the initial attack of a collagenolytic MMP. Mesenchymal cell types such as fibroblasts, osteoblasts, some endothelial cells, and some macrophages express uPARAP/Endo180, with a strong expression in areas with dominant collagen turnover, such as developing bone. PyMT mice, which develop genetically induced, invasive mammary tumors, have reduced tumor growth and increased tumor collagen content when uPARAP/Endo180 is absent due to gene inactivation. Cancer cells have not been found to express uPARAP/Endo180 but some of the same cell types that express the receptor in healthy tissue show a strong increase in expression when they take part in the stroma that surrounds the cancer islets in some invasive cancers. Thus, in some situations involving invasive growth, collagen clearance by uPARAP/Endo180 is likely to take active part in the outgrowth and escape of cancer cells from a confined tissue compartment.

After the submission of this manuscript, it has been reported that uPARAP/Endo180 is expressed in basal-like breast cancer cells (Wienke, D. et al. (2007) Cancer Res. 67:10230–10240).

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Identification of uPARAP/Endo180

At the protein level the endocytic receptor uPARAP/Endo180 was identified by two independent groups. In one study, the protein was identified in a trimolecular complex by the use of cross-linking experiments with prourokinase and the urokinase-type plasminogen activator receptor (uPAR), on the surface of U937 cells (Behrendt et al. 1993). This observation indicated that the protein was situated in close proximity to uPAR on the surface of U937 cells in order for the cross-linking to take place, thus giving rise to the name uPAR-associated protein (uPARAP). A tryptic digest of the cross-linked complex was subjected to mass spectrometry-based analysis, and the protein was sequenced and cloned and proved to be a novel member of the macrophage mannose receptor family (Behrendt et al. 2000). This protein family consists of four members: the macrophage mannose receptor (MMR), the secretory phospholipase A2 receptor (PLA2R), the receptor DEC-205, and uPARAP (Behrendt 2004).

In an independent work, an unknown protein of molecular weight 180 kDa was observed by use of monoclonal antibodies obtained after immunization of mice with intact, or membrane fractions of, human fibroblasts (Isacke et al. 1990), and this protein was subsequently cloned and shown to be identical with uPARAP (Sheikh et al. 2000). The antibody in question was reactive with a cell-surface protein occurring on several cultured cell types including fibroblasts, macrophages, and endothelial cells. Moreover, the protein showed evidence of endocytosis with internalization into endosomes via clathrin-coated pits and recycling to the plasma membrane; thus the name Endo180 was chosen (Sheikh et al. 2000). In the following, the designation uPARAP/Endo180 will be used.

It should be noted that the uPARAP/Endo180 encoding cDNA was identified already in 1996 in a third, independent work (Wu et al. 1996), although no characterization of the protein was performed at that point.

Protein Structure

Based on sequence alignment with the macrophage mannose receptor, the domain structure of uPARAP/Endo180 could be deduced. From the extracellular amino terminus, uPARAP/Endo180 consists of a Cys-rich domain followed by a fibronectin type-II (FN-II) domain, eight C-type carbohydrate recognition domains (CRDs), a transmembrane segment, and a cytoplasmic domain (Behrendt et al. 2000, Sheikh et al. 2000). The crystal structure of uPARAP/Endo180 has not yet been determined but three-dimensional structures have been determined for two domain types in the presumably similar MMR. These are the MMR Cys-rich domain (Liu et al. 2000) and the MMR CRD4 (Feinberg et al. 2000).

Cys-Rich Domain

The Cys-rich domain of the MMR is a globular structure with a β-trefoil as the structural basis, where three similar β-sheets, each composed of four strands, are organized in a near symmetric manner (Liu et al. 2000). The MMR Cys-rich domain binds sulfated sugars in a loop of six amino acids between β-strand 11 and 12, but this loop is absent from the Cys-rich domains of uPARAP/Endo180 and the other members of the MMR protein family. Thus, the Cys-rich domain of uPARAP/Endo180 probably does not bind sulfated sugars (Liu et al. 2000, East and Isacke 2002).

FN-II Domain of uPARAP/Endo180

The FN-II domain structure has not been determined for any member of the MMR family, but the sequence similarity in this region is high between the MMR family members and there is also a large degree of homology with FN-II domains of proteins outside the MMR family (East and Isacke 2002, Wienke et al. 2003). Because of this large degree of homology, it seems reasonable to assume that the known structure of, for example, the third FN-II domain of MMP-2, a domain important for the collagen binding ability of this collagenolytic enzyme, has some similarity with the FN-II domain structure of uPARAP/Endo180 (Behrendt 2004). The former FN-II domain is a compact structure with two double-stranded, antiparallel β-sheets oriented at a right angle to each other (Briknarova et al. 2001). A large region of the exposed surface is a hydrophobic region that binds to peptides mimicking gelatin (Briknarova et al. 2001). FN-II domains are known to take part in collagen binding in several proteins (Banyai et al. 1990) and this prompted a competition experiment with various collagens in the above- mentioned cross-linking setup with pro-uPA/uPAR and uPARAP/Endo180. This experiment demonstrated a robust inhibition of the pro-uPA–uPARAP/Endo180 cross-linking with collagen type V and a moderate inhibitory effect of collagen types I and IV (Behrendt et al. 2000), thus providing the first indication for a collagen-binding function of uPARAP/Endo180. An open question relates to the notion that, in various other collagen-binding proteins, two or more FN-II domains may be required for an efficient binding to collagen (Banyai et al. 1994, Pickford et al. 1997) whereas only one FN-II domain is present in uPARAP/Endo180. On the contrary, an NMR study on a structure including two FN-II domains of MMP-2 revealed little interaction between these domains (Briknarova et al. 2001) and, thus, it is uncertain whether there is indeed a requirement for more than one FN-II domain for collagen binding in the latter protein. Even if the single FN-II domain of uPARAP/Endo180 is not sufficient for efficient binding to collagen, several mechanisms could contribute to compensate for this. Thus, a clustering of several uPARAP/Endo180 molecules is not an unlikely event, as the protein is already concentrated in areas of clathrin-coated pits, and since the collagen itself could aid in the clustering process as one weak collagen binding could be stabilized with the binding of more uPARAP/Endo180 molecules.

Furthermore, as support for the ability of proteins with a single FN-II domain to bind collagen, studies on the closely related MMR (Napper et al. 2006. Martinez-Pomares et al. 2006) show binding to collagen via the FN-II domain independently of CRDs.

CRDs

In several proteins, domains with sequence homology with the CRDs of uPARAP/Endo180 are involved in carbohydrate binding. This is exemplified by one or two CRDs in the homologue MMR. Just like the latter protein, uPARAP/Endo180 includes eight putative CRDs. However, in uPARAP/Endo180 only CRD2 has retained the structural elements for chelating critical Ca^{++} ions involved in the carbohydrate-binding mechanism of this domain type (Sheikh et al. 2000, East and Isacke 2002). In accordance with this notion, probably only CRD2 has carbohydrate-binding activity (East et al. 2002). The structure of a typical sugar-binding CRD is stabilized by two disulfide bonds and has an organized core with two α-helices and two β-sheets, of which one has two strands and the other has three (East and Isacke 2002), and with a loop extending from the largest β-sheet. The actual binding to sugars is dependent on two Ca^{++} ions in a hydrophobic fold, where two coordination sites exist. One Ca^{++} ion is directly involved in sugar binding (the principal site) and the other functions as a structural support of the hydrophobic part of the CRD (East and Isacke 2002).

A series of affinity columns containing different immobilized sugars were tested for binding of recombinant, soluble uPARAP/Endo180. Whereas no binding was found to mannose, fucose, or galactose, uPARAP/Endo180 could be bound to and eluted from a column containing *N*-acetylglucosamine-agarose (East et al. 2002). So far, however, no biological ligand has been found for the lectin function of uPARAP/Endo180.

Cytoplasmic Domain

The cytoplasmic domain of uPARAP/Endo180 contains two motifs that were initially both considered candidates for governing the endocytic function: a tyrosine-based endocytosis motif (Tyr1452) and a di-hydrophobic (Leu1468-Val1469) endocytosis motif with a negatively charged residue (Glu1464) being positioned 4 amino acids prior to the motif (Sheikh et al. 2000, Howard and Isacke 2002). In MHC class II proteins, a di-hydrophobic motif, similar to that found in uPARAP/Endo180, has been shown to target internalized vesicles to early endosomes (Pond et al. 1995). A mutagenesis experiment revealed that substitution of the tyrosine motif does not interfere with internalization (Howard and Isacke 2002). In contrast, mutagenesis of the di-hydrophobic motif to Ala-Ala led to a strong decrease in internalization and mutation of the −4 glutamate also led to a reduction (Howard and Isacke 2002). The mechanism behind uPARAP/Endo180 receptor recycling from early endosomes back to the plasma membrane is still unclear, as a di-aromatic motif important for receptor

recycling of MMR and PLA2R is not present in uPARAP/Endo180 (East and Isacke 2002). uPARAP/Endo180 is a constitutively active internalization receptor; however, it has been suggested that some regulation of the internalization process takes place through phosphorylation of serine residues adjacent to the endocytosis motif. Thus, a low level of serine phosphorylation was observed in immunoprecipitated uPARAP/Endo180 protein from human fibroblasts, with a strong increase in phosphorylation being found in phorbol ester-treated cells (Sheikh et al. 2000).

Interdomain Organization

Analysis of the spatial domain organization of uPARAP/Endo180 by single particle electron microscopy with recombinant uPARAP/Endo180 variants has led to a model in which the Cys-rich domain folds back to contact CRD2 (Rivera-Calzada et al. 2003). A later study supports this notion and furthermore suggests the possibility of a pH-dependent conformational change with a more open conformation of the backfold at acidic pH (Boskovic et al. 2006). It is tempting to speculate that this type of conformational change has a function in the release of bound, internalized collagen in a more acidic endosomal compartment (Boskovic et al. 2006), where uPARAP/Endo180 enters during the constitutive recycling of the receptor (Sheikh et al. 2000).

uPARAP/Endo180-Deficient Mice

Mice with genetic uPARAP/Endo180 deficiency were generated using two strategies, leading to very similar genetic constructs. In both cases, a complete functional deficiency was obtained in terms of collagen interactions although, as detailed below, certain targeted cells in culture may express low levels of truncated uPARAP/Endo180 antigen. In one study, the targeting strategy included a construct leading to deletion of the entire region containing exons 2–6. An RNA transcript of the targeted uPARAP/Endo180 DNA was seen expressed in fibroblasts from newborn uPARAP/Endo180 targeted mice, but no protein expression could be detected at that point in these animals (Engelholm et al. 2003). However, later work has suggested that low levels of a truncated protein with a size in accordance with the stated deletion of exons may indeed be present in some cultured cells from these mice (D.H. Madsen, the Finsen Laboratory, unpublished observation). In another study, exons 2–5 and part of exon 6 were deleted. In the targeted mouse embryos, low levels of a truncated protein of molecular weight 140 kD could be identified (East et al. 2003). Thus, these truncated proteins would be lacking the Cys-rich domain, the FN-II domain, and the first CRD. In the study by East et al., it was positively demonstrated that the protein product retained the sugar-binding properties previously reported to originate from CRD2, whereas the collagen-binding function was lost (East et al. 2003). In both studies, the mice deficient in uPARAP/Endo180 were viable with no obvious aberrant phenotype and were able to reproduce (East et al. 2003, Engelholm et al. 2003). However, a recent detailed study on

bone development, performed with the mice generated by Engelholm et al., has revealed a small retardation in the growth of long bones and cranial closure (Wagenaar-Miller et al. 2007).

Collagen Internalization

As detailed above, a binding to collagen was noted already in the early characterization of uPARAP/Endo180 (Behrendt et al. 2000). The ability of the receptor to internalize collagen was subsequently demonstrated for collagens I, IV, and V in a study using radioactively labeled collagens (Engelholm et al. 2003). Newborn mouse fibroblasts from wild-type and uPARAP/Endo180-deficient animals were incubated with 125I-labeled collagens for determination of collagen internalization. Strikingly, whereas wild-type fibroblasts displayed an efficient uptake of collagen, the uPARAP/Endo180-deficient fibroblasts were completely unable to perform this process, thus identifying uPARAP/Endo180 as a crucial collagen internalization receptor (Engelholm et al. 2003).

In another study, the ability of uPARAP/Endo180 to internalize collagen was likewise demonstrated with fibroblasts from uPARAP/Endo180 +/+ and −/− mice, but utilizing an assay where cells were incubated with fluorescence-labeled collagen or gelatin (East et al. 2003). Fibroblasts from the uPARAP/Endo180-targeted mice were unable to bind collagen type IV and gelatin, and also unable to internalize these proteins (East et al. 2003). Binding to and internalization of collagen by uPARAP/Endo180 was also demonstrated in a study using both soluble, recombinant purified uPARAP/Endo180 and cell-based experiments (Wienke et al. 2003). The receptor bound to the different collagens tested (collagen types I, II, IV, and gelatin), and in a competition experiment, collagen IV and gelatin could mutually compete the binding of each other. Furthermore, binding to collagens was unaffected in engineered uPARAP/Endo180 proteins without CRDs 5–8, and also maintained for all tested collagen types (except collagen IV) in a protein without CRDs 2–8 (Wienke et al. 2003). Cells transfected with uPARAP/Endo180 (MCF-7 and T47D breast carcinoma cells) and cells expressing endogenous uPARAP/Endo180 (MG-63 osteosarcoma cells) were shown to bind to collagen IV and gelatin, and this binding was blocked by uPARAP/Endo180 siRNA treatment (Wienke et al. 2003). Furthermore, mutation of the intracellular dihydrophobic internalization motif resulted in unaltered collagen binding, but absence of collagen internalization (Wienke et al. 2003). The subcellular fate of the internalized collagen was investigated in fibroblasts from newborn mice (Kjoller et al. 2004). Treatment with E64D, an inhibitor of lysosomal cysteine proteases, resulted in a strong accumulation of vesicles containing collagen, giving an indication that collagen is directed to lysosomes. This observation was confirmed by costaining with the lysosomal marker Lamp-1 (*see* Fig. 13.1; Kjoller et al. 2004). In mouse tissue explants, the uptake and intracellular degradation of collagen was seen in uPARAP/Endo180-expressing chondrocytes and osteoblasts of developing bone (Wagenaar-Miller et al. 2007). A recent study with mouse fibroblasts showed that

the efficiency of uPARAP/Endo180 to internalize collagen was increased when the collagen was precleaved into defined large fragments by a collagenase (Madsen et al. 2007). The improved efficiency was a result of the cleaved collagen attaining a gelatin-like state at physiological temperature. Thus, heat-denatured intact collagen was likewise internalized more efficiently, whereas heat-denatured collagen fragments were internalized to the same degree as nonheated fragments. The importance of this observation was studied by analyzing culture media from uPARAP/Endo180-containing and uPARAP/Endo180-deficient fibroblasts grown on a collagen matrix. It was evident that there was an accumulation of collagen fragments in the media from uPARAP/Endo180-deficient cells but not in the media from uPARAP/Endo180-positive cells. This observation provided a strong indication of a sequential mechanism of collagen degradation by fibroblasts, with initial cleavage by collagenolytic metalloproteases and subsequent clearance of collagen fragments through a uPARAP/Endo180-dependent internalization route (Madsen et al. 2007). The whole process is then finalized by intracellular degradation by lysosomal proteases.

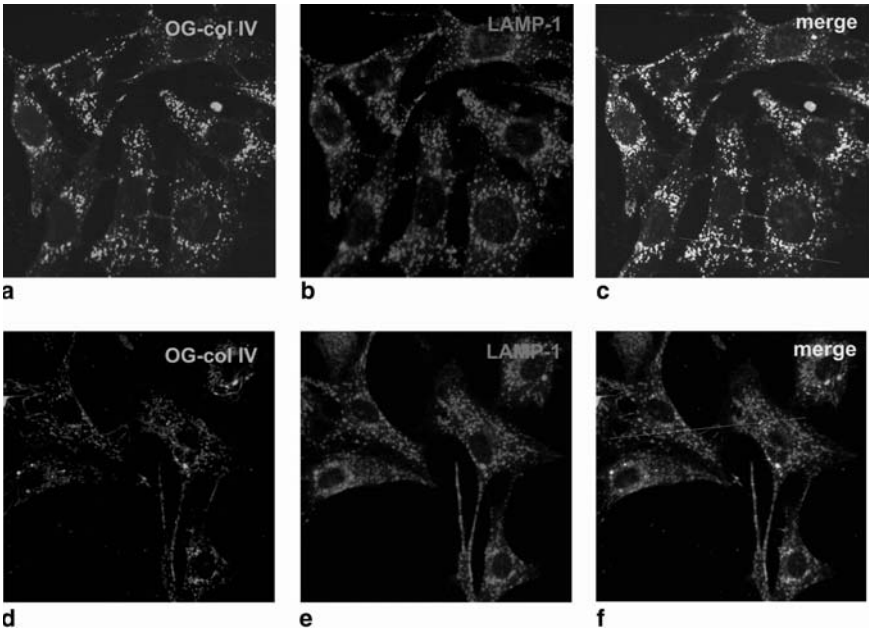

Fig. 13.1 Internalized collagen is directed to lysosomal degradation. Wild-type (**a–c**) and *uPARAP/Endo*180-deficient (**d–f**) fibroblasts were treated with an inhibitor of lysosomal cysteine proteases, E64d. Cells were then incubated with Oregon green-labeled collagen IV for 16 h at 37°C. Lysosomes were labeled with red fluorescence, using a LAMP-1-specific antibody. A strong lysosomal accumulation of OG-collagen is seen in wild-type fibroblasts, whereas cells deficient for uPARAP/Endo180 do not internalize collagen. (Reproduced from Kjoller et al. 2004 with permission from the publisher) (*See also* Color Insert I)

A recent work has demonstrated that the related MMR is also a collagen clearance receptor. This receptor is strongly expressed by liver sinusoidal endothelial cells and was found to be responsible for approximately half of the liver retention of collagen fragments from plasma (Malovic et al. 2007). The remaining half was also cleared by livers from MMR-deficient mice. The latter observation was not accounted for, but could be a result of other MMR family members also being expressed in the liver.

uPA-System Interaction

On certain types of cultured cells, for example U937 cells, uPARAP/Endo180 is located in close proximity to uPAR on the cell surface [shown in cross-linking experiments (Behrendt et al. 2000)]. The physical intimacy between the proteins led to speculation regarding a cooperative pathway of matrix degradation, initially by envisioning uPARAP/Endo180 as a receptor that brought the plasminogen activation event and downstream collagenolytic enzymes in close proximity to matrix substrates (Behrendt et al. 2000). Later, as the internalization properties of the protein were discovered, this hypothesis was refined to represent the intracellular component of a matrix-degrading machinery (Engelholm et al. 2003). It is unclear if such a cooperative complex exists in vivo in areas of tissue remodeling. Many cell types that are uPARAP/Endo180 positive probably express little or no uPAR but in some cases, such as the osteoblasts of developing bone, a strong expression of both receptors is indeed noted (Engelholm et al. 2001).

Role of uPARAP/Endo180 in Cell Migration

The generation of mice deficient for uPARAP/Endo180 enabled experiments with murine fibroblasts to determine a possible role of the receptor in motility and migration. Fibroblasts from uPARAP/Endo180-deficient mice were found to be less motile than those from uPARAP/Endo180-expressing littermate wild-type control cells, on both matrigel and rat tendon collagen matrices (East et al. 2003, Engelholm et al. 2003). The mechanistic background for this phenomenon is still unresolved, but could possibly include a stronger adhesion provided by uPARAP/Endo180, thus providing a firmer grip on the matrix by the migrating fibroblasts and resulting in more efficient movement. Also, a more indirect effect of uPARAP/Endo180 on migration is possible as a putative interplay with integrins could result in signaling events, giving rise to increased motility. In another set of studies, the directional migration against a uPA gradient was found to be inhibited in a uPARAP/Endo180 si-RNA-treated breast cancer cell line (Sturge et al. 2003). The directional sensing was maintained in cells expressing a uPARAP/Endo180 variant which was mutated in the di-hydrophobic internalization motif (Sturge et al. 2003), whereas no sense of direction was observed after treatment of the

cells with antibodies directed against two of the four outermost domains of uPARAP/Endo180 (CRD2 and the Cys-rich domain) (Sturge et al. 2003). The results were interpreted as evidence for a specific uPARAP/Endo180-mediated regulation of uPA-uPAR-induced cellular orientation. As the orientation effect was independent of internalization, it is possible that a better adhesion in uPARAP/Endo180-expressing cells contributed to the effects observed. This also might be consistent with the above-mentioned results with blocking antibodies directed against domains close to the FN-II domain responsible for collagen binding. A more recent study by the same investigators, examining the involvement of uPARAP/Endo180 in migration, indicated that the pathway including Rho, Rho-kinase (ROCK), and myosin light chain 2 (MLC2) phosphorylation was necessary for rear cell deadhesion during cell migration, and that this pathway was dependent on the uPARAP/Endo180-containing endosome compartment (Sturge, Wienke, and Isacke 2006).

Histological Localization

uPARAP/Endo180 in Healthy Tissue

The expression of uPARAP/Endo180 has been observed in several types of mesenchymal cells such as fibroblasts, osteoblasts, certain endothelial cells, and some macrophages in various organs, including developing bone, placenta, bladder, skin, kidney, spleen, liver, and lung (Isacke et al. 1990. Wu et al. 1996, Sheikh et al. 2000, Engelholm et al. 2001, Mousavi et al. 2005, Honardoust et al. 2006). Tissue undergoing remodeling of its collagen matrix often shows expression of uPARAP/Endo180 in some cell populations. In gingival wounds, a strong upregulation was noted in several cell types including myofibroblasts, macrophages, and endothelial cells (Honardoust et al. 2006). Furthermore, uPARAP/Endo180 is strongly expressed in the developing bones of mouse embryos and newborn mice (Wu et al. 1996, Engelholm et al. 2001, Howard et al. 2004, Wagenaar-Miller et al. 2007). In this case, the protein is expressed by osteoblasts and chondrocytes (Engelholm et al. 2001, Wagenaar-Miller et al. 2007). By screening for uPARAP/Endo180 mRNA expression in several tissues, high levels were found in lung and kidney, whereas the liver and brain were found to be almost negative (Wu et al. 1996), although with expression of an alternatively spliced, truncated RNA in fetal liver. In apparent contradiction to a lack of expression in the liver, hepatic stellate cells in culture have been shown to internalize collagen in a uPARAP/Endo180-dependent manner (Mousavi et al. 2005). As stellate cells comprise only 10% of the cells in the liver, an expression in these cells may have been difficult to detect in the study by Wu et al. Another region reported to be without uPARAP/Endo180 is the epidermis, whereas dermal macrophages have been shown to be uPARAP/Endo180 positive (Sheikh et al. 2000).

uPARAP/Endo180 in Cancer

In a recent study, uPARAP/Endo180 deficiency was combined with the MMTV-PyMT mouse breast tumor model in which mice develop spontaneous malignant breast tumors. Mice deficient for uPARAP/Endo180 showed a significantly delayed primary tumor growth and strikingly, the tumors had a very high content of undegraded collagen compared to the tumors of the littermate uPARAP/Endo180-positive controls (Curino et al. 2005). Moreover, no intracellular collagen was found in explanted fibroblast-like cells from the tumors of uPARAP/Endo180-deficient mice, in contrast to what was seen in the uPARAP/Endo180-sufficient cells. The expression of uPARAP/Endo180 in the tumors from wild-type PyMT mice mimicked that found in the human disease (see further below), with expression in fibroblast-like cells surrounding the mammary ducts and no expression in tumor cells (Nielsen et al. 2002, Curino et al. 2005). It was concluded that collagen clearance by intracellular degradation via the uPARAP/Endo180-dependent pathway was involved in invasive tumor growth in this system.

A study of uPARAP/Endo180 protein and mRNA expression (Nielsen et al. 2002) showed only very little expression in the normal human breast, with detectable staining only in a few fibroblast-like or myoepithelial cells. In benign breast lesions, uPARAP/Endo180 staining was strongly positive in intralobular fibroblasts and, depending on the type of lesion, also in myoepithelium and tumor-associated fibroblasts. In ductal carcinoma in situ (DCIS; *see* Fig. 13.2), uPARAP/Endo180 was observed in both myoepithelial cells and some tumor-associated fibroblasts, with more uPARAP/Endo180-positive fibroblasts around some of the DCIS foci. Invasive carcinomas of both ductal and lobular type also showed uPARAP/Endo180 staining of fibroblast-like cells, whereas the cancer cells were uPARAP/Endo180 negative in all cases. The pattern of staining by immunohistochemistry was indistinguishable from the pattern obtained by in situ hybridization (Nielsen et al. 2002). Similarly, in a study of 112 human squamous cell carcinomas of the head and neck, uPARAP/Endo180 showed increased expression especially in fibroblast-like cells from the tumor stroma (Sulek et al. 2007). Whereas no case of expression of uPARAP/Endo180 in cancer cells has been reported so far*, the total expression pattern is nevertheless in complete accordance with a function in cancer. In all cases studied, uPARAP/Endo180 is expressed in fibroblast-like cells in close proximity to the tumor and in some cases as part of the enveloping stroma barrier (Nielsen et al. 2002). It is indeed plausible that uPARAP/Endo180 promotes the mechanism of invasion and escape of tumor cells in the local tissue compartment, based on the ability of the receptor to assist collagen degradation and clearance. It is noteworthy that, in a study of colorectal cancer, searching for tumor endothelial markers by expressed sequence tags, uPARAP/Endo180 was identified as one of the most upregulated genes in endothelial cells adjacent to the cancer compared to endothelial cells from normal colorectal tissue

* Please refer to note added in proof on the first page.

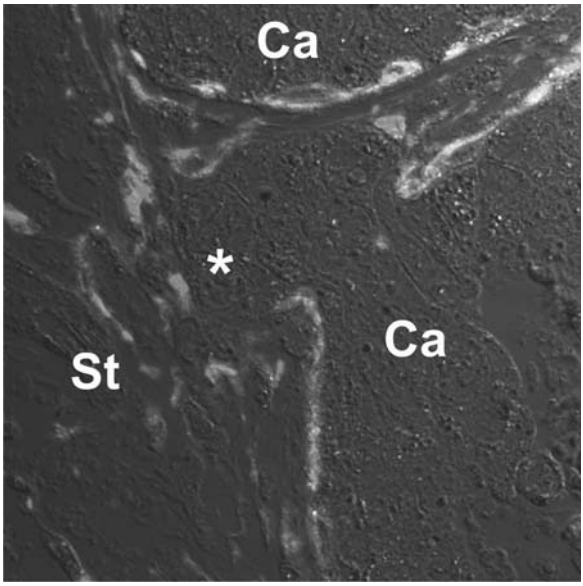

Fig. 13.2 Expression of *uPARAP*/*Endo*180 at a tumor–stroma interface. Double immunostaining of uPARAP/Endo180 (rabbit antibody coupled to Cy3; red) and cytokeratin 17 [myoepithelial marker, monoclonal mouse antibody coupled to fluorescein isothiocyanate (*FITC*); green] in human breast ductal carcinoma in situ with focal invasion (indicated by star). uPARAP/Endo180 is expressed in fibroblasts and some myoepithelial cells surrounding the carcinoma cells (*Ca*) and in fibroblasts located in the stroma (*St*). For material and methods, see Nielsen et al. (2007). (Boye Schnack Nielsen, the Finsen Laboratory, Copenhagen, unpublished work; figure kindly made available by B.S. Nielsen) (*See also* Color Insert I)

(St Croix et al. 2000). It was not completely clear, however, if fibroblast-like cells may have contributed to the cell populations analyzed in this study.

Biological Role of uPARAP/Endo180 and Concluding Remarks

Even though the knowledge on the function of uPARAP/Endo180 in development and in different tissues is still very incomplete, the local expression patterns and analyses of the biochemical properties of the protein do provide strong suggestions as to some roles of the receptor in vivo. As noted above, the uPARAP/Endo180 mRNA is expressed in developing bone from newborn mice and mouse embryos (Wu et al. 1996, Engelholm et al. 2001), and the cellular origin of the in situ signal is most likely the osteoblast-osteocyte and possibly also osteoclasts and endothelial cells (Engelholm et al. 2001, Wagenaar-Miller et al. 2007). Moreover, uPARAP/Endo180 expression has been observed in cartilage-forming sites in young mice (Howard et al. 2004). All of these observations are compatible with the hypothesis that uPARAP/Endo180 has a role in collagen turnover in developing tissue. This is consistent with the notion that although mice deficient in uPARAP/Endo180 appear

normal and are fertile (East et al. 2003, Engelholm et al. 2003), a close examination of the developing bone did reveal a small delay in the time-course of bone growth in the uPARAP/Endo180-deficient, newborn mouse (Wagenaar-Miller et al. 2007). In the same study, however, this became even much more clear when uPARAP/Endo180 deficiency was combined with deficiency for MT1-MMP, a membrane-bound protease involved in collagen degradation which is expressed in areas of collagen turnover in developing bone, overlapping with those of uPARAP/Endo180 expression (Wagenaar-Miller et al. 2007). Mice deficient for MT1-MMP alone were small and affected in bone and connective tissues, with arthritis and osteopenia and reduced survival, and with ~30% of mice dying before weaning and the remaining before 90 days of age (Holmbeck et al. 1999, Wagenaar-Miller et al. 2007), but in the study on combined deficiency, these conditions were severely worsened. Although MT1-MMP; uPARAP/Endo180 double-deficient mice were born in the expected mendelian ratio, they were uniformly prone to early postnatal death, with all mice dying within the first 21 days of age. The development of bone was heavily retarded, including the calvarial bone, and the cranial closure was very incomplete in the double-deficient mice (Wagenaar-Miller et al. 2007).

Altogether the above observations give strong indications pointing to uPARAP/Endo180 being physiologically relevant in several remodeling events involving collagen degradation. Importantly, extracellular matrix degradation/remodeling is a crucial step in cancer invasion (Hanahan and Weinberg 2000), and therefore the above-mentioned role of uPARAP/Endo180 in the mouse tumor model (Curino et al. 2005) is most likely related to the same basic functions as those relevant to the healthy remodeling processes. The likely importance of uPARAP/Endo180 in invasion makes it a candidate therapeutic target in cancer treatment, aiming to prevent the spread of malignant disease.

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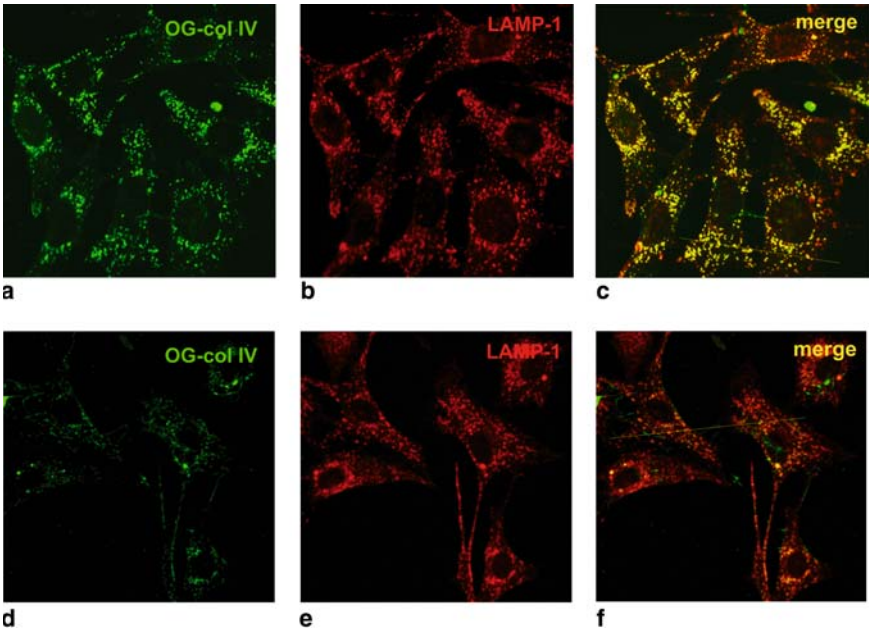

Fig. 13.1 Internalized collagen is directed to lysosomal degradation. Wild-type (**a–c**) and urokinase-type plasminogen activator receptor associated protein (*uPARAP*/*Endo*180)-deficient (**d–f**) fibroblasts were treated with an inhibitor of lysosomal cysteine proteases, E64d. Cells were then incubated with Oregon green-labeled collagen IV for 16 h at 37°C. Lysosomes were labeled with red fluorescence, using a LAMP-1-specific antibody. A strong lysosomal accumulation of OG-collagen is seen in wild-type fibroblasts, whereas cells deficient for uPARAP/Endo180 do not internalize collagen. (Reproduced from Kjoller et al. 2004 with permission from the publisher).)

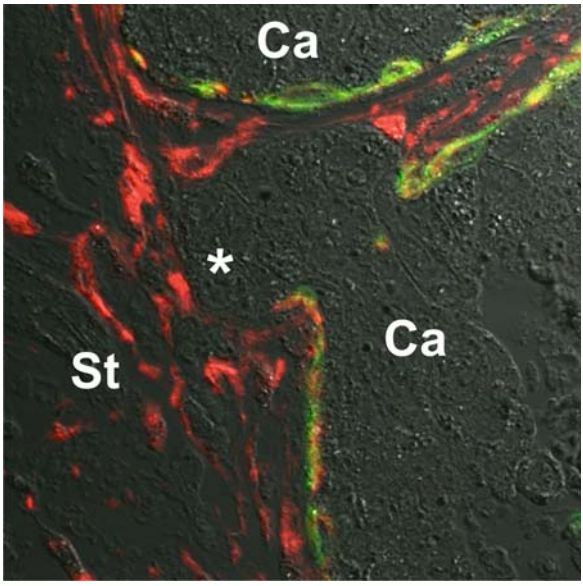

Fig. 13.2 Expression of urokinase-type plasminogen activator receptor associated protein (*uPARAP*/*Endo*180) at a tumor–stroma interface. Double immunostaining of uPARAP/Endo180 (rabbit antibody coupled to Cy3; red) and cytokeratin 17 [myoepithelial marker, monoclonal mouse antibody coupled to fluorescein isothiocyanate (*FITC*); green] in human breast ductal carcinoma in situ with focal invasion (indicated by star). uPARAP/Endo180 is expressed in fibroblasts and some myoepithelial cells surrounding the carcinoma cells (*Ca*) and in fibroblasts located in the stroma (*St*). For material and methods, see Nielsen et al. (2007). (Boye Schnack-Nielsen, the Finsen Laboratory, Copenhagen, unpublished work; figure kindly made available by B.S. Nielsen).