[Frontiers in Bioscience 14, 2103-2114, January 1, 2009]

The collagen receptor uPARAP/Endo180

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1. ABSTRACT

The uPAR-associated protein (uPARAP/Endo180), a type-1 membrane protein belonging to the mannose receptor family, is an endocytic receptor for collagen. Through this endocytic function, the protein takes part in a previously unrecognized mechanism of collagen turnover. uPARAP/Endo180 can bind and internalize both intact and partially degraded collagens. In some turnover pathways, the function of the receptor probably involves an interplay with certain matrix-degrading proteases whereas, in other physiological processes, redundant mechanisms involving both endocytic and pericellular collagenolysis seem to operate in parallel. On certain cell types, uPARAP/Endo180 occurs in a complex with the urokinase plasminogen activator receptor (uPAR) where it seems to fulfill other functions in addition to collagenolysis. uPARAP/Endo180 is expressed on various mesenchymal cells, including subpopulations of fibroblasts, osteoblasts and chondrocytes, generally in conjunction with matrix turnover and collagenolysis. A striking expression is found in developing bone where the collagenolytic function of uPARAP/Endo180 is one of the rate-limiting steps in growth. In murine breast tumors, the endocytic function of the receptor in collagen breakdown seems to be involved in invasive tumor growth.

2. INTRODUCTION

The uPAR-associated protein (uPARAP/Endo180; other designations: CD280 or MRC2), is an endocytic receptor with a crucial role in the turnover of collagen by mesenchymal cells. This receptor, in the following just designated uPARAP, binds directly to collagen and also interacts, through an unknown mechanism, with components of the PA system. The present review will be focused primarily on the function of uPARAP in collagenolysis, whereas additional functions related to interactions with other molecules will be treated more briefly. The description will also address a number of aspects related to the expression of uPARAP in healthy and diseased tissues and the physiological importance of the protein, including its role in cancer.

3. PROTEIN STRUCTURE AND MOLECULAR INTERACTIONS

3.1. uPARAP belongs to an established protein family of endocytic receptors

uPARAP is a type-1 membrane protein of M_r ~180,000 with an amino acid sequence indicating that it belongs to the macrophage mannose receptor (MRC1) protein family (1-3). In addition to uPARAP, this family

Tabel 1. The mannose protein receptor family

NCBI database name	Synonyms	Accession no. for human cDNA
Mannose receptor, C type 2 (MRC2)	uPARAP, Endo180, CD280, MRC2	NM_006039
Mannose receptor, C type 1 (MRC1)	MR, CD206	NM_002438
Secretory phospholipase A2 receptor (PLA2R)	PLA2R, PLA2-R, PLA2IR, FcRY	NM_001007267
Lymphocyte antigen 75 (LY75)	DEC-205, GP200-MR6, CD205	NM_002349

includes the mannose receptor (MR) itself (4), the secretory phospholipase A_2 receptor (PLA_2R) (5, 6), and an endocytic protein which is designated DEC205 or gp200-MR6 (7, 8)(see table 1 for further information). The chicken PLA_2R functions as a yolk sac IgY receptor (9). All of the proteins in this family are endocytic receptors and share a highly characteristic domain composition as detailed in the following section. (5, 6).

3.2. The domains of uPARAP

The extracellular part of the members of this protein family includes three well-defined domain types which are organized in the same manner in all members (10, 11). Thus, from the N-terminus, each of these proteins is built up with a single ricin B-like / cystein-rich (CysR) domain, a single fibronectin type-II (FnII) domain and a series of C-type lectin-like domains (CTLDs) which, except for short inter-domain regions, comprise all of the rest of the extracellular part. There are eight CTLDs in all of the family members except for DEC205/gp200-MR6 which has ten. The CTLDs are followed by a transmembrane segment and a small cytoplasmic domain (Figure 1).

In spite of this similarity, the proteins of this family have distinct biological roles, dictated by different ligands and different patterns of tissue expression. Nevertheless, some functions seem to be shared, or at least related, between some or all of the members. It is also evident that, although a number of ligands have been assigned to members of this protein family already, new binding partners and new functions of these domain types are still likely to emerge.

With respect to the CysR domain, a function has been identified only in the case of MR where this domain binds to negatively charged (e.g. sulfated) oligosaccharides (12-14). Structural considerations suggest that the CysR domains of the other family members, including uPARAP, do not share this function (10, 15) and neither has any alternative ligand been assigned to this domain type in these proteins.

The FnII domain is considered central for the collagen-binding function of uPARAP and early work, as well as more recent studies, suggest that this function may be shared between the protein family members (16-19). This function of uPARAP will be covered in detail in the following sections.

The CTLDs, in spite of their lectin-like sequence properties, probably only take part in specific carbohydrate-binding reactions in a minority of cases. The most thoroughly studied example is the macrophage mannose receptor which binds to oligosaccharides or protein carbohydrate side chains with terminal mannose, fucose, and N-acetylglucosamine residues, the interaction occurring through the CTLD-4 or the sum of CTLD-4 and CTLD-5 (20, 21). Carbohydrate-binding reactions have also been demonstrated in the case of uPARAP (2, 3) where the active domain appears to be CTLD-2 (15). However, in spite of a well defined carbohydrate specificity as found with artificially immobilized sugars (15), no biological ligand has been ascribed to the lectin function of uPARAP so far. Therefore, this binding capability will not be treated thoroughly in this review.

So far, no data are available with respect to the three-dimensional structures of the individual domains of uPARAP. Crystal structures have been solved for the CysR domain of MMR (14) and the CTLD-4 of the same protein (22) however the latter structure does probably not represent the active carbohydrate-binding conformation. Since the crystallized CTLD-4 did not contain the active consensus structure with two Ca^{2+} ions and was unable to incorporate an oligosaccharide in a co-crystallization experiment. In addition, 3-dimensional structures have been solved for various domains of members of related protein families which are assumed to share the over-all folding topology with the domains of the present protein family. The latter examples include the NMR structure of an Fn-II domain in MMP-2 (23) and the ligand binding domain of MBP-A, complexed with an oligosaccharide (24). A discussion of structural features of some of these related protein domains can be found in previous review articles (10, 11).

3.3. The interaction of uPARAP with collagen

uPARAP binds directly to native and partially denatured collagen. This collagen-binding activity was first recognized in competition experiments in conjunction with cross-linking studies with uPARAP and pro-urokinase. Thus particularly collagen type V was able to compete the cross-linking between uPARAP and pro-uPA very efficiently in the applied setup (2). This interaction is treated later in this review.

The FnII domain of uPARAP is considered central for the collagen-binding function (review: (11)). Nevertheless, the details of the role of the domain composition in the collagen interactions are still not known. A recombinant product comprising just the first three domains of uPARAP (CysR, FnII and CTLD-1; Figure 1) has been shown to retain collagen binding (25) and, similarly, no collagen binding was found with a truncated protein lacking the same domains but including the rest of the protein (26). Studies on the gross molecular shape and the steric domain organization, using single particle electron microscopy, revealed a bent shape of the

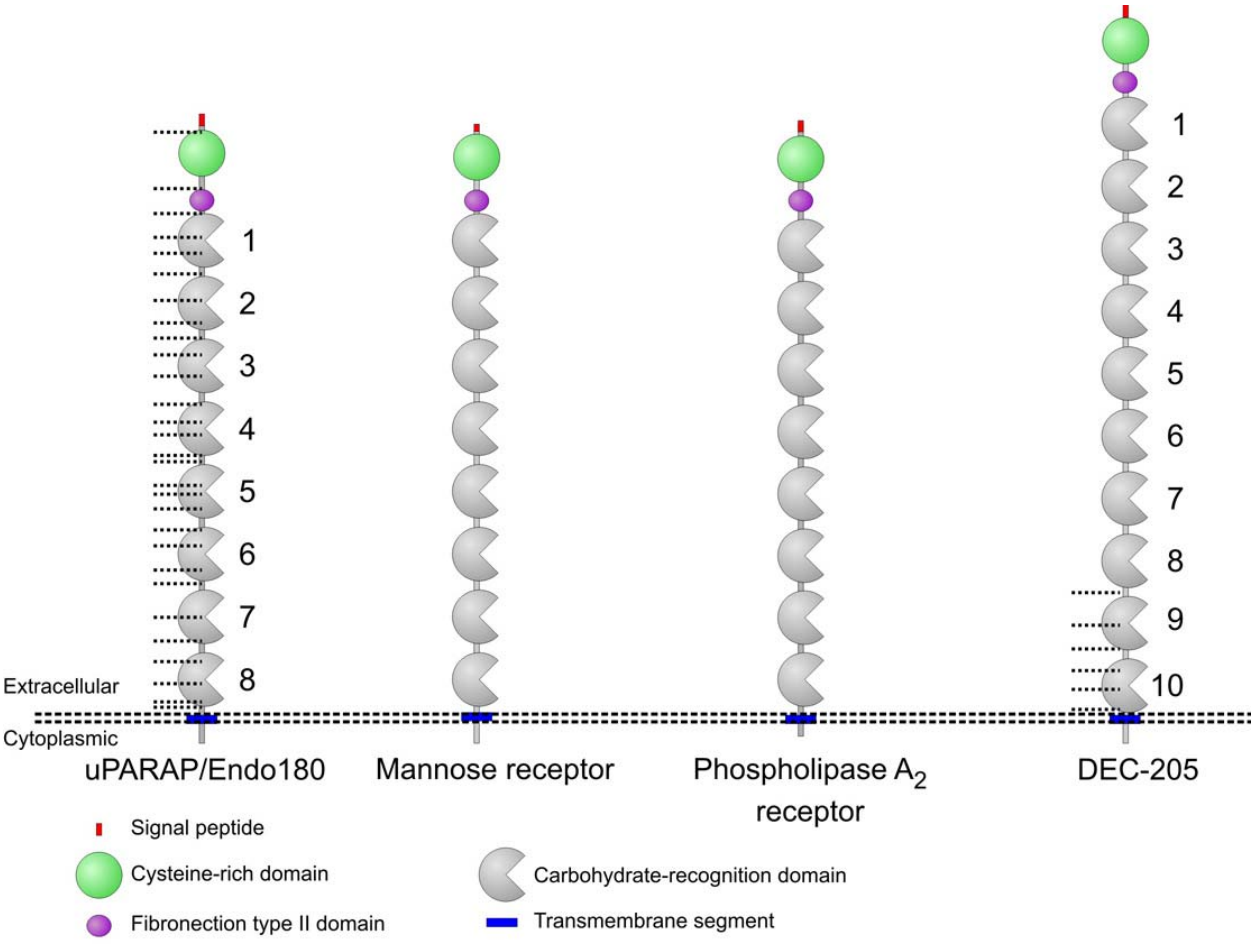

Figure 1. The Mannose receptor family. All of the family members have the indicated domain composition starting with a cysteine-rich domain in the N terminal part, followed by a fibronectin type II domain and 8 to 10 carbohydrate recognition domains, a transmembrane spanning region and a small cytoplasmic tail. Intron/exon boundaries are indicated by dotted line in uPARAP/Endo180. These boundaries are conserved throughout the receptor family, with two additional CRDs in DEC-2005 most likely due to a gene duplication of CRD 7 and 8 (53).

extracellular part of uPARAP in which the N-terminal CysR domain contacts the second CTLD (27). The formation of this contact creates a globular "head", most distant from the plasma membrane, in which the FnII domain and the first CTLD are exposed with a fixed relative orientation. Importantly, this contact is disrupted under low pH conditions, resulting in a more open conformation, leading to the hypothesis that pH dependent shifts in the relative domain organization may govern ligand binding and release (28). This latter property may be central to the function of uPARAP as a constitutively recycling internalization receptor; see further below. A more complete evaluation of the functional role of the steric domain arrangement must, however, await structural studies at a higher resolution.

With respect to the binding partner, early studies suggested that uPARAP has a preference for some collagen subtypes, notably collagen V (2). More recently, however, it has been demonstrated that the subtle transition to a "gelatin-like" state that occurs in collagens upon collagenase-mediated cleavage leads to increased binding to uPARAP (Figure 2) (29). This transition includes a partial denaturation and, therefore, it is possible that variations in the native state between purified preparations of the different collagen subtypes account for some of the differences noted with respect to binding to uPARAP. Whereas a complete evaluation of the binding preferences must await studies with defined, native fibrillar and sheet-oriented collagens, as well as their cleaved or denatured equivalents, studies with purified collagens have so far revealed binding and /or internalization with at least collagen I, IV and V (2, 30, 31). At this point it seems most likely that the potential for uPARAP-mediated binding and uptake is common to most or all collagens.

As detailed below, uPARAP-mediated endocytosis can be part of an integrated pathway of

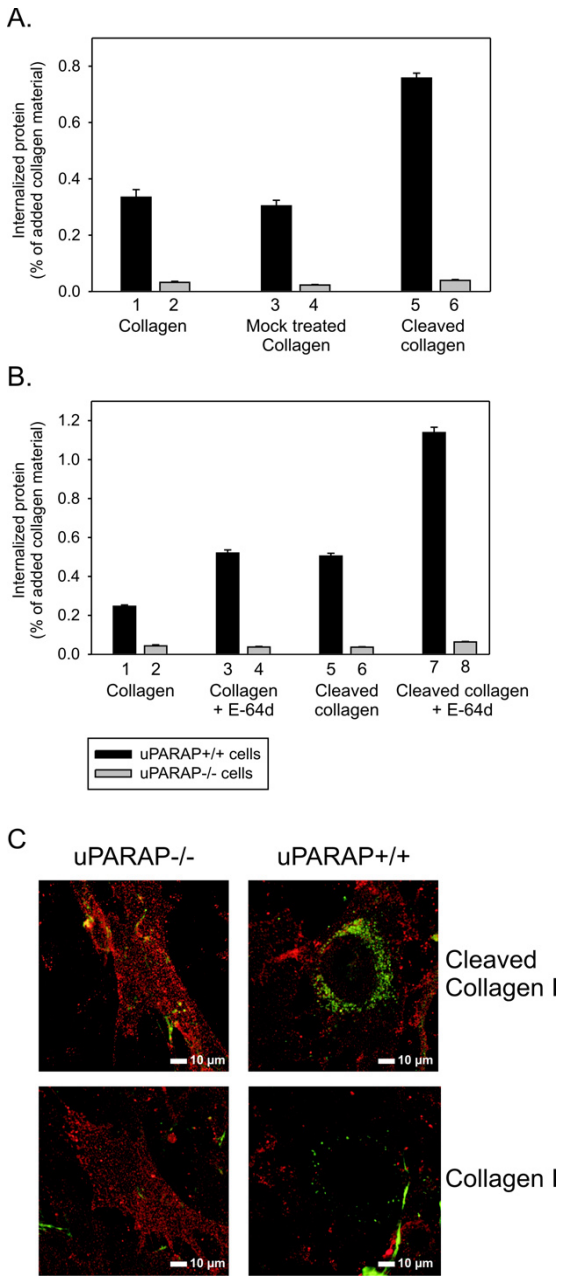

Figure 2. uPARAP-dependent internalization of labeled collagen and collagen fragments. A, Increased uptake after cleavage. 125I-labeled untreated, mock-incubated or cleaved collagen type-I (30 ng/well) was added to fibroblast cultures derived from wildtype (uPARAP-expressing) mice (+/+, black columns) or from littermate mice with homozygous uPARAP deficiency (-/-, grey columns). After 3 h of incubation at 37°C, the intracellular fraction of each cell sample was isolated and the amount of internalized labeled protein was determined by gamma counting. All samples were analyzed in triplicate. The data are represented as the internalized fraction of the added radioactivity; note that the same amount of protein material was added to all samples. Error bars indicate standard deviations. B, Internalized collagen fragments are routed to intracellular degradation. Fibroblast cultures were incubated with ^{125}I-labeled intact or cleaved collagen type-I as in A, except that incubation was performed in the absence or presence of 20 µM of E-64d, as indicated. The protease inhibitor leads to a strong increase in the intracellular accumulation of collagen material. C, Demonstration of intracellular fluorescence-labeled collagen fragments following uPARAP-mediated endocytosis. FITC-labeled collagen type Icleaved with collagenase-3 (two upper slides) or intact, FITC-labeled collagen (two lower slides) was added to fibroblast cultures derived from wild-type (right slides) or littermate uPARAP-deficient mice (left slides), after which the cells were cultured for 22 h in the presence of E-64d. The cells were examined by confocal microscopy. Adapted from (29) with permission from the American Society for Biochemistry.

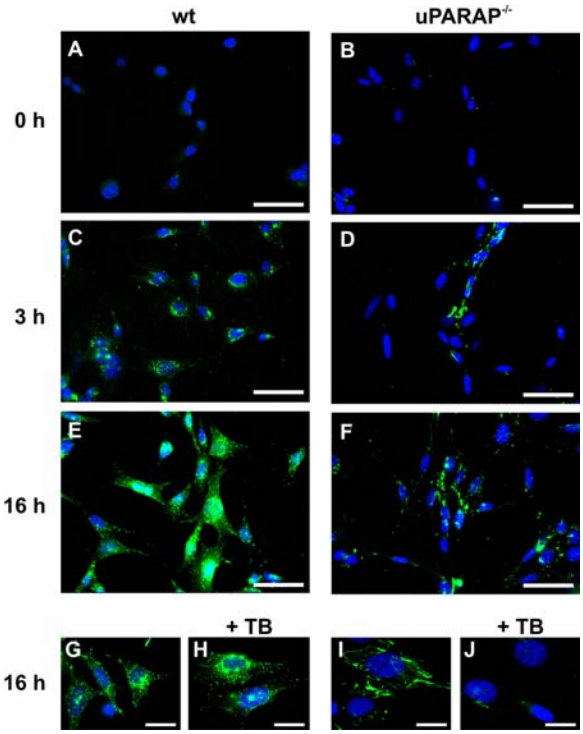

Figure 3. Localization of fluorescent collagen IV after addition to wt (A, C, E) and uPARAP/endo180 -/- (B, D, F) fibroblasts. Cells were preincubated with OG-collagen IV (green fluorescence) at 4°C, transferred to 37°C (for details see (31)) and incubated for 0 h (A, B), 3 h (C, D) or 16 h (E, F, G, H, I, J) at 37°C. Cells were then washed, fixed and counterstained with DAPI (blue) to visualize nuclei. In some cases (H, J), cells were treated with trypan blue to quench extracellular fluorescence prior to fixation. Note the exclusive accumulation of intracellular collagen in uPARAP positive cells Bar: 50 µm (A, B, C, D, E, F) or 20 µm (G, H, I, J). Reproduced with permission from Elsevier Limited.

collagen turnover and, as mentioned already, uPARAP reacts efficiently with pre-cleaved collagen (29). Mammalian collagenases perform their attack on triple-helical collagen at a single, defined position, leading to the formation of "3/4" and "1/4" fragments. Studies with collagen fragments generated in cell culture suggest that uPARAP is reactive with both of these fragments (as analyzed in the case of collagen type-I) (29). A different study, addressing the binding to a recombinant collagen fusion protein, showed that uPARAP can bind to a region in the C-terminal part of the collagen I α1 chain (32); i.e., a region included in the ¼ fragment. This, however, did not exclude the additional binding to other molecular regions because intact collagen was not investigated in the study.

4. THE CELLULAR FUNCTION OF UPARAP

4.1. Collagen turnover

The function of uPARAP most obviously connected with matrix turnover is the role of the protein in collagen degradation. This function will be given the major emphasis here.

Studies by gene inactivation revealed that fibroblasts isolated from uPARAP-/- mice are unable to internalize collagen (26, 30). Furthermore, a comparison between the uPARAP-/- fibroblasts and cells from littermate wildtype mice made it clear that the collagen taken up by the wildtype cells was routed to lysosomal degradation, a function which was thus ablated in the uPARAP-deficient cells (31) (see Figure 3). This ability of uPARAP-expressing cells to internalize collagen and gelatin and to route this material to lysosomes was also demonstrated with uPARAP-transfected MCF7 breast cancer cells (25).

The actual function of uPARAP in an integrated collagen turnover mechanism could be demonstrated in long term cell culture studies with uPARAP-/- cells. When grown on a reconstituted native collagen I matrix, fibroblasts gradually solubilize this matrix through the action of matrix metalloproteases (MMPs) that serve to cleave the collagen into ¼ and ¾ fragments. However, whereas wildtype fibroblasts succeeded to clear the resulting fragments from the culture supernatant, thus completing the degradation process, uPARAP-deficient fibroblasts turned out to accumulate these fragments in the supernatant as a result of defective internalization and lysosomal breakdown (29).

The complete mechanism of collagen breakdown via the endocytic route has not yet been worked out. So far, however, it has been shown that uPARAP itself is constitutively and rapidly recycling between clathrin-coated pits on the cell surface and the early endosomal compartment (33, 34). When ligated with a monoclonal antibody, cell surface uPARAP was thus internalized with a half-life of a few minutes at 37°C (34) and at any time point, the major part of the cellular pool of uPARAP seems to be situated intracellularly (33).

The properties of the small, cytoplasmic domain of uPARAP have been studied with respect to the endocytic function. Mutation of the "dihydrophobic" motif, Leu^{1468}-Val^{1469}, into Ala-Ala led to an internalization-defective receptor (34) that, consequently, failed to undertake the endocytic routing of collagen/gelatin to intracellular vesicles (25). This dihydrophobic motif is probably involved in the interaction with "adaptor complexes" to allow clathrin-mediated internalization (34). Whereas the intracellular trafficking signals of this system are not clear, it has been suggested that phosphorylation of serine residue(s), observed in purified uPARAP, may play a role in this context (3).

Importantly, in the case of collagen endocytosis, this mechanism implicates a dissociation event in the endosomal compartment from which the receptor is recycled to the cell surface whereas collagen ligands are routed further to late endosomes and lysosomes. In the actual collagen degradation process, it is likely that lysosomal cysteine proteases (35-37) play a major role

because the specific inhibition of these enzymes leads to lysosomal entrapment and accumulation of internalized collagen (31).

An important, still open question related to the function of uPARAP in collagen turnover is whether this receptor can take part in a phagocytic uptake event. In one study adressing this question, uPARAP-transfected cells failed to internalize polystyrene beads coated with a monoclonal antibody against the receptor. Since the cell types in question were indeed capable of phagocytosis in a positive control system with different beads with the same physical properties, this observation disfavoured a phagocytic mechanism connected with uPARAP (34). However, as also pointed out by those authors, this does not exclude an interplay of uPARAP with a phagocytic machinery under other conditions. Indeed, studies on the role of uPARAP in collagen turnover in the stroma of murine mammary tumors suggested a uPARAP-mediated uptake of particulate collagen, based on the observation of uPARAP-dependent accumulation of intracellular, banded collagen as revealed by electron microscopy (38). This raises the question how uPARAP engages collagen *in vivo*. Obviously there must be a size limit as to how large collagen particles can be taken up by cells and routed to intracellular degradation. Thus, one must image that a partial cleavage of the native collagen fibres has to occur before a subsequent uPARAP mediated processing can take place. The details of this uptake are still an open field and will await future studies to clarify the precise mechanism.

4.2. Interaction with pro-uPA and uPAR

On certain cell types, uPARAP can take part in a ternary complex with pro-urokinase and the urokinase receptor, uPAR, on the plasma membrane. After addition of radiolabeled pro-uPA to human monocyte-like U937 cells, enzymatic cross-linking with tissue transglutaminase was used to demonstrate a labelled pro-uPA-uPARAP complex on the cell surface (39). Combination of this method and conventional cross-linking with N,N´-disuccinimidylsuberate (an amino group-reactive, homobifunctional reagent) led to demonstration of a trimolecular complex including both pro-uPA, uPAR and uPARAP (2). However, the details of this reaction are not clear. Thus, the mere co-expression of uPAR and uPARAP on a certain cell type is not necessarily sufficient to allow the formation of the ternary complex with pro-uPA and, at least so far, BIAcore-based experiments have failed to demonstrate an interaction between the three constituent proteins in a purified system (our unpublished results). Apparently, certain subtle but specific arrangements of the involved components on the cell surface need to be fulfilled for efficient complex formation, or the reaction may be dependent on as-yet unidentified co-factors. Furthermore, since the demonstration of this interaction relies on covalent cross-linking, the lifetime of the non-covalent complex on the cell surface has not been determined.

The functional implications of this complex formation are not fully known. However, a signaling mechanism with importance for directional cell migration, demonstrated in human MDA-MB-231 cells which express both uPARAP and uPAR, was found to be sensitive to siRNA mediated knock-down of uPARAP expression (40). This directional migration, which occurred specifically against a uPA gradient, could also be blocked by a monoclonal antibody directed against the CTLD-2 of uPARAP but did not require the endocytic activity of the intracellular domain. In addition, however, expression of uPARAP was found to stimulate the non-directional motility of the same cell type on various matrices and in contrast to the directional migration, this was due to a mechanism that was indeed dependent of the endocytic function of the receptor (40). The mechanism lying behind the latter observation was suggested to include a function of endosomal uPARAP in stimulating adhesion disassembly, mediated by ROCK- (Rho-associated kinase) dependent contractile signals (41).

Additional roles of the interplay between uPARAP and the plasminogen activation system could be more directly related to proteolytic reactions, although any such models are still speculative. It has been suggested that, through the association with uPAR, uPARAP may serve to present collagen substrates close to the site where collagenolytic enzymes become activated (2, 42). The latter activation reactions could include plasmin-mediated activation of MMP-1 and/or MMP-13, this plasmin activity originating from uPA-catalyzed plasminogen activation, occurring on uPAR-expressing cells. Alternatively, or in addition, uPARAP might serve to internalize collagen fragments originating from cleavages performed by the same collagenases as mentioned above, again as a result of uPAR- and uPA-dependent plasminogen activation.

5. TISSUE EXPRESSION AND PHYSIOLOGICAL FUNCTIONS OF uPARAP

5.1 Cellular and tissue specific expression

uPARAP is predominantly expressed on cells of mesenchymal origin. In healthy, normal tissues the receptor is expressed on several cell types, among which various subsets of fibroblasts and osteogenic cells show a particularly strong expression. In many cases, the sites of expression are connected with growth or tissue remodeling.

In mouse embryos and young mice, the bone forming regions display a particularly dominant expression of uPARAP. In a study on mouse embryos by *in situ* hybridization, the receptor seemed to be expressed in all tissues undergoing primary ossification. Interestingly, these areas of expression included both sites of intramembranous ossification (the viscerocranium and calvarium) and sites of endochondral ossification (the developing long bones). The primary uPARAP-positive cell types appeared to be cells of the osteoblast-osteocyte lineage (42). In the developing bone of young mice, studied postnatally, uPARAP was expressed by osteoblasts/osteocytes and by resting, reserve, and proliferating zone chondrocytes whereas no expression was observed in hypertrophic chondrocytes and endothelial cells (43). The expression of uPARAP in cartilage has also

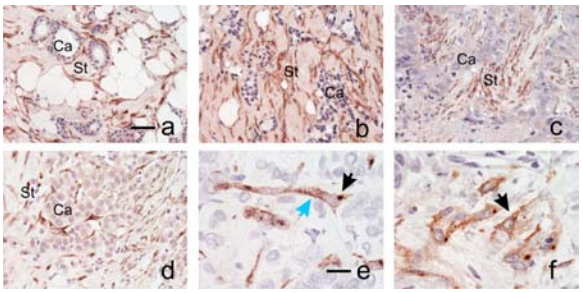

Figure 4. Immunohistochemical staining of uPARAP in invasive ductal and lobular human breast carcinomas. Sections from invasive ductal (a-c, e and f) and invasive lobular (d) carcinoma were immunohistochemically stained with affinity purified polyclonal antibodies against uPARAP. uPARAP immunoreactivity is seen in stromal cells (St) surrounding the cancer cells (Ca) that are all negative. Note the intense perinuclear uPARAP immunoreactivity (black arrow in e), the granulated cytoplasmic localization (blue arrow in e), and the localization suggesting an association of the protein with the cell surface (arrow in f). Scale bars, a-d: 50 µm, e-f: 25 µm. Reproduced from (47) with permission from Wiley Interscience.

been studied in young mice. A strong expression was noted in chondrocytes in the articular cartilage, with this expression decreasing with the age of the animals (44).

In the human placenta, a dominant expression of uPARAP was found in mesenchymal cells in the villous stroma whereas trophoblastic cells are uPARAP negative (45). Also, the endothelium of the larger microvessels of the placenta has been shown to express uPARAP (3) whereas, in most other cases studied, endothelial cells seem to be uPARAP negative.

In the human skin, the epidermis was shown to be uPARAP negative and the expression of the receptor in this tissue was limited to dermal macrophages (3). In marked contrast to the relative scarcity of uPARAP-positive cells in the skin, human gingiva was found to include several uPARAP-positive cell types (46). Notably, in this particular tissue the basal epithelial cells showed positive immunohistochemical staining for uPARAP and the protein was also observed on fibroblasts, myofibroblasts, endothelial cells, pericytes and macrophages. The same work included a study on the uPARAP-expression during gingival wound healing. In this case, uPARAP was observed on migrating epithelial cells and on various subsets of the same additional cell types as found positive in the unwounded gingiva but with a varying expression level, reflecting the different healing stages.

In the normal, human breast, uPARAP is expressed on some myoepithelial cells and intralobular and interlobular fibroblasts, although the abundance of positive cells and the expression level have been evaluated somewhat differently in the studies performed (47, 48). Part of this apparent discrepancy may be due to a comparison with the situation in malignant breast lesions in which a strong upregulation occurs (47); see further below.

Several cultured cell types also express uPARAP. These include malignant cell types such as MG-63 osteosarcoma cells (25, 33), monocyte-like U937 cells (2, 39), HT1080 fibrosarcoma cells (25) and MDA-MB-231 breast tumor cells (40, 48) but also primary cultures of fibroblasts (30), hepatic stellate cells (49), osteoblasts (43) and chondrocytes (43, 44).

5.2. Expression of uPARAP in cancer

Due to the presumed function of uPARAP in extracellular matrix degradation, special emphasis has been given to the expression of the receptor in connection with invasive tumors.

Indeed, although evidence from this area of investigation is just beginning to accumulate, it is evident that a strong expression of uPARAP occurs in the sites of cancer invasion studied so far and that this represents a strong increase in expression relative to neighbouring, normal tissue. However, in accordance with the predominant mesenchymal expression found in normal tissues, in epithelial cancers the expression is most often restricted to the stromal compartment, rather than to the cancer cells themselves (Figure 4).

In human squamous cell carcinoma of the head and neck, uPARAP was observed on a subset of stromal cells with fibroblast-like morphology (50). The expression in these cells was clearly increased relative to the more distal, tumor-adjacent submucosa, as well as relative to healthy specimens from the same tissue location. The same study included a tissue array-based analysis of a large number of cases (112 tumors) which were scored with respect to uPARAP occurrence as well as immunostaining intensity. This analysis confirmed the localization and upregulation, noted above, as being a dominant pattern, although the tissue arrays also included a minority of tumor specimens that did not show uPARAP expression above the detection limit.

A comparison of different tumor grades revealed that the most prominent expression occurred in the stroma of poorly differentiated tumors (50).

In human ductal carcinoma *in situ* of the breast, a strong upregulation of uPARAP, relative to the more distant, "normal" tissue, was observed in myoepithelial and tumor-associated fibroblast-like cells (47). In invasive breast carcinoma, analyzed in the same study, a strong uPARAP staining was found mainly in tumor-associated myofibroblasts and to a lesser extent in macrophages. In all of the cases studied in this work (i.e., a total of 21 invasive ductal and lobular carcinoma), the cancer cells were uPARAP-negative (47). More recently, however, a tissue array-based study of a larger material has been analyzed (48). In by far the majority of cases, the expression pattern noted above was confirmed but interestingly, in 3-6 % of

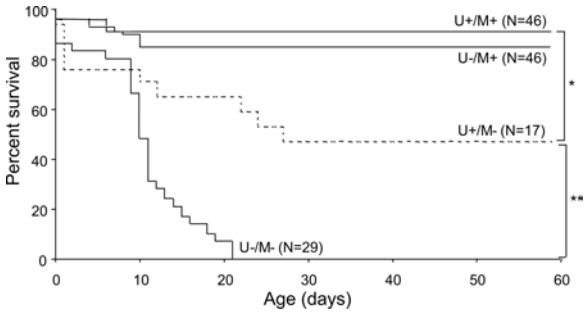

Figure 5. Combined loss of uPARAP/Endo180 and MT1-MMP causes early postnatal death. FVB mice with targeted uPARAP/Endo180 and MT1-MMP alleles were interbred to generate uPARAP/Endo180 and MT1-MMP-sufficient (M+/U+), uPARAP/Endo180-deficient (U-/M+), MT1-MMP-deficient (U+/M-), and uPARAP/Endo180 and MT1-MMP double-deficient (U-/M-) littermate offspring. (A) 60-day survival of a prospective cohort initially consisting of 46 U+/M+, 46 U-/M+, 17 U+/M-, and 29 U-/M- mice. All U-/M- mice perished before day 21, while the 60-day survival of U+/M+ mice was 91 %, survival of U-/M+ mice was 85 %, and survival of U+/M- mice was 47 %. * = $P < 0.0012$. ** = $P < 0.007$, Mann-Whitney U-test, two-tailed. Adapted from (43) with permission from the American Society for Microbiology.

the specimens (depending on the tissue arrays under study), a strongly positive uPARAP signal was observed in cancer cells. The tumors in question belonged to the basal type breast cancers, a relatively rare type of breast cancer, and only a subgroup of these cancers showed uPARAP-positive tumor cells. This low abundance of positive samples probably explains the lack of similar observations in the previous study. The expression of uPARAP in tumor cells in this particular type of cancer is in line with the notion that the general protein expression pattern in basal type breast cancer appears to be more closely related to that of the myoepithelial cells of the normal breast than to that of the luminal epithelial cells (48).

5.3. Physiological function of uPARAP and phenotype of uPARAP-deficient mice

uPARAP gene-targeted mice have been created in two independent studies (26, 30), using mutually similar over-all strategies. The targeted portion of the gene included exon 2-5 and in addition either the whole or part of exon 6. The deletion of this gene segment leads to loss of the part encoding the CysR, the FnII and the CTLD-1 domain. It may be noted that this genetic construct does not necessarily exclude the formation of a truncated protein product in the gene targeted mice since a splice product can theoretically be formed, connecting exon 1 with exon 7. In the study by East et al., a truncated protein product (comprising CTLD2 – 8, the transmembrane part and the cytoplasmic domain) could indeed be demonstrated on cultured fibroblasts from gene targeted mouse embryos. Analyses with fibroblasts from the mice generated by Engelholm et al. have suggested a low expression of a similar product (our unpublished results), although a complete evaluation has not been performed in this case. Nevertheless, any such truncated product is completely devoid of collagen binding since the deleted, N-terminal fragment is completely necessary for this interaction (see above).

In both studies, the uPARAP-targeted mice were born in a normal Mendelian ratio, proved viable, had normal survival and were fertile (26, 30). Also, upon gross inspection, no phenotypic abnormalities were apparent. However, a recent study has revealed a slight but significant delay in bone growth in the homozygous gene-targeted mice (43) and furthermore, the uPARAP deficiency has an effect on tumor growth in a mouse model system; see further in the following sections.

Since uPARAP is a central player in the endocytic pathway of collagen turnover as shown *in vitro*, the relatively low physiological effect of uPARAP gene targeting suggests that additional turnover pathways are operative *in vivo*. When pursuing this question, an interesting candidate protein for a redundant function in collagenolysis is the membrane-bound collagenase, MMP-14. This protease has been shown to be highly important for processes depending on collagen cleavage *in vivo* (51). Consequently, in a recent study the gene deficiencies for uPARAP and MMP-14 were combined to learn about the overlapping pathways operative in collagen turnover. A strong synergistic effect was observed in this experiment. Mice deficient for MMP-14 alone have reduced survival, with only about half of the mice surviving throughout the experimental period of 60 days. When combined with uPARAP deficiency, however, this was severely worsened, with all double-deficient mice dying before three weeks of age (43) (Figure 5). Both MMP-14 and uPARAP are expressed in developing bone and gene targeting of either component alone had a measurable effect on bone growth (51); see also above. When focusing specifically on the bone of the young, double deficient mice, however, even more severe defects were observed. The growth of the long bones was delayed more strongly than in the single-deficient mice and the closure of the craniae was found to be very incomplete (43).

5.4. Role in cancer

The localization of uPARAP in tumor tissue and the functional role of the receptor as studied *in vitro* have prompted investigations of a potential role of uPARAP in invasive cancer growth. The availability of uPARAP-targeted mice thus enabled a study on the effect of this functional deficiency on the growth of genetically induced malignant breast tumors. uPARAP deficient mice were interbred with transgenic mice carrying the PymT oncogene under the control of the MMTV promoter (PymT mice) which develop spontaneous, invasive breast tumors (38). In the tumors of uPARAP positive PymT mice, the protein was found to be expressed in a pattern closely resembling that of the majority of human malignant breast tumors, with a strong upregulation in fibroblast-like cells in the stromal compartment, and with the cancer cells being devoid of uPARAP expression. In the uPARAP-targeted

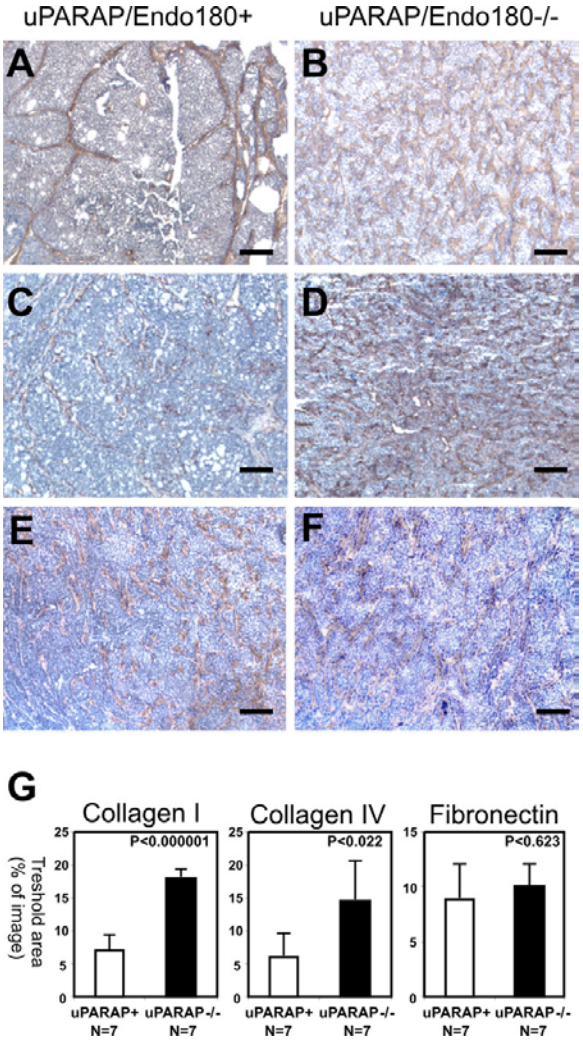

Figure 6. Collagen accumulation in uPARAP/Endo180-deficient mammary tumors. Representative examples of the appearance of uPARAP/Endo180^{+} tumors (A, C and E) and uPARAP/Endo180$^{-/-}$ tumors (B, D and F) after immunohistochemical staining for collagen type I, (A and B), type IV (C and D)or fibronectin (E and F). uPARAP/Endo180$^{-/-}$ tumors display obvious accumulation of collagen in the tumor stroma surrounding nests of tumor cells, while no difference between the genotypes, is apparent in fibronectin deposition around tumor cell nests. Bars; 500 μm. (G) Histomorphometric quantitation of collagen I, collagen IV and fibronectin accumulation in uPARAP/Endo180^{+} (white boxes) and uPARAP/Endo180$^{-/-}$ (black boxes) tumors. All P values were determined by Student's t-test, two-tailed. Adapted from (38) with permission from Rockefeller University Press.

mice, the growth of the tumors turned out to be significantly delayed relative to the uPARAP-expressing littermates. Whereas this difference was only modest, a much more striking effect was noted when comparing the collagen content of the tumors. Thus, in the uPARAP-targeted mice a strong accumulation of collagen was noted in areas of the tumor stroma, surrounding nests of tumor cells, whereas no such accumulation was observed in the tumors of the uPARAP-positive mice (38) (Figure 6).

Whereas this study addressed the importance of stromally expressed uPARAP, another recent study has focused on the functional role of uPARAP when expressed on cancer cells (see also above). When human MCF7 breast cancer cells were transfected with uPARAP-encoding cDNA and inoculated into immunocompromised mice, the resulting tumors grew more rapidly and displayed a smaller collagen content than tumors formed by mock-transfected cells (48). Interestingly, transfection with an endocytosis-defective uPARAP mutant protein did not have the same effect, thus underscoring the importance of the endocytic function of uPARAP for collagen turnover and promotion of tumor growth (48).

6. CONCLUSIONS AND PERSPECTIVES

Experiments performed so far to elucidate the structure and function of uPARAP have pointed to a central role in collagenolysis in conjunction with tissue remodeling and cancer, although additional functions are also apparent. A number of molecular and cellular features important for the understanding of these roles of the protein have already been uncovered.

Extensive studies are still needed, however, to learn more about the collagen interactions of uPARAP, notably including investigations on the three-dimensional structure of the collagen-binding elements. Furthermore, an important area of research will be the possible identification of additional ligands with relevance *in vivo* and the unravelling of the interplay with uPAR and the plasminogen activation system.

Future work should also include studies to learn about the physiological routes of collagen breakdown in which uPARAP takes part, including the serial and parallel pathways in which uPARAP can either cooperate with, or be substituted by, other components. The identification of these reaction partners and components with overlapping function will be central to any attempts to interfere therapeutically with collagenolysis and matrix turnover. To this end, an important issue will be the role of the related proteins in the mannose receptor protein family since it is becoming increasingly clear that, within this family, interactions with collagen are not restricted to uPARAP (16-19). Thus, a tempting hypothesis could be that the MR protein family members play a general role in collagen turnover, depending on their sites of expression, as supported by recent studies on combined deficiency for uPARAP and MMR (52). Also, additional studies should focus on collagen-degrading MMPs and cysteine proteases.

In relation to the role of uPARAP in disease, much more work is needed to obtain a complete picture of the localization of uPARAP in various cancers and in other diseases, such as arthritis. Very importantly, this has to be supplemented with a number of model studies in mice

which will be necessary to learn about the causal role of uPARAP-mediated reactions in those conditions.

7. ACKNOWLEDGEMENTS

This work was supported by EU contract LSHC-CT-2003-503297 and by grants from the Danish Cancer Society, the Danish Cancer Research Foundation and the Danish Medical Research Council.

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Key Words: Collagen receptor, Internalization Receptor, Intracellular Collagen Degradation, Collagenolysis, Matrix Metalloproteinases, Endocytic Pathway, Lysosomal Collagen Breakdown, Review

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