

uPAR is over-expressed with good correlation between the expression of uPAR and the invasiveness/metastatic potential of the cancer. One important factor is that uPA/uPAR focalises the conversion of plasminogen to plasmin. Plasmin then digests fibrin extracellular matrix (ECM) and this subsequently facilitates metastasis and invasion. Plasmin has also been shown to activate precursors of matrix metalloproteinases which are also involved in ECM degradation which would be expected to potentiate the effect (Murphy & Gavrilovic, 1999).

The expression of uPA and PAI-1 also seems to be increased in several cancers (De Petro et al., 1998, Gavrilov et al., 2001, Mori et al., 2000, Morita et al., 1998). uPA has also been shown to release fibroblast growth factor 2 (FGF2) from the ECM (Ribatti et al., 1999), and this can be cleaved by matrix metalloproteinase 9 to form angiostatin (Patterson & Sang, 1997). The formation of angiostatin from uPA shows adroit control over the angiogenic/angiostatic mechanism and the complex interplay between molecules in the body. The non-catalytic amino terminal fragment was not found to be angiogenic as it does not release FGF2 from the ECM (Ribatti et al., 1999). Binding of uPA to uPAR provokes a mitogenic response as does the amino terminal fragment and cyclic uPA₁₉₋₃₁ (disulphide bridge between cysteines, CVS NKYFSNIHWC) in human ovarian cancer cells (Fischer et al., 1998). The cell model used was treated with antisense against uPA to reduce the auto/paracrine effect on cells and the cyclic peptide was added at a 12x higher molar concentration than uPA, these factors may have contributed to the observed increase in cell proliferation. The amino terminal fragment was not found to induce proliferation of breast cancer cells (8701-BC cells) (Luparello & Del Rosso, 1996).

1.4.3 Interactions of Membrane Components with uPAR

GPI-linked receptor internalisation is poorly understood in comparison with transmembrane receptors. However, internalisation of uPAR proceeds after recruitment of various cell membrane components. The endocytosis of uPAR has been observed through several different mechanisms which are summarised in Fig. 1.9. Both clathrin-mediated and non-clathrin mediated endocytosis of uPAR have been reported (Vilhardt et al., 1999). Clathrin-mediated endocytosis has been more widely studied and characterised (Slepnev & Camilli, 1998).

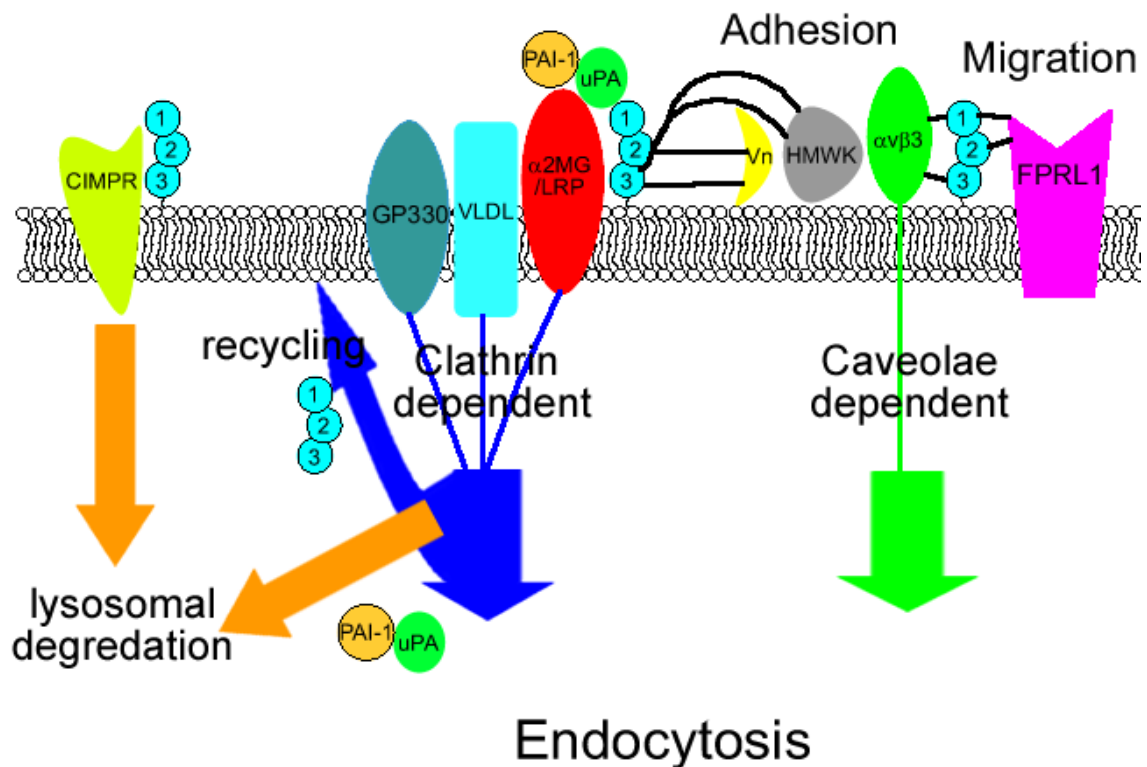


Figure 1.9 - Co-receptors that interact with uPAR

Key: CIMPR – cation independent mannose-6-phosphate receptor; GP330 – glycoprotein 330; VLDL – very low density lipoprotein receptor; α 2MG/LRP – α 2-macroglobulin receptor/lipoprotein related receptor; Vn – vitronectin, HMWK – high molecular weight kallikrine; α v β 3 – α v β 3 integrin; FPRL1 – formyl like peptide receptor (Compiled from: Andreasen et al., 2000, Blasi & Carmeliet, 2002, Brown & London, 1998, Czekay et al., 2001, Moestrup et al., 1993, Nykjaer et al., 1998, Nykjaer et al., 1997, Nykjaer et al., 1994a)

Clathrin is a protein found on the cytosolic side of the cell. It consists of three heavy chains (180 kDa) and three light chains (~35 kDa) (Stryer, 1995). These chains come together to form a three legged structure, a triskelion, which forms into a lattice and creates a coating around endocytic vesicles as they form (Stryer, 1995). The release of this polyhedral lattice constructed from pentagons and hexagons formed from triskelions of clathrin is facilitated by an adenosine triphosphate (ATP)-driven uncoating enzyme (Stryer, 1995).

The low density lipoprotein receptor family are most heavily implicated in the endocytosis of uPAR. These receptors are commonly found in lipid rafts (Brown & London, 1998) and are endocytosed in clathrin coated pits. This family includes low density lipoprotein related protein (LDLRP) (Czekay et al., 2001), α -2-macroglobulin/lipoprotein receptor protein (Nykjaer et al., 1994a), very low density lipoprotein receptor (VLDL) (Nykjaer et al., 1997) and glycoprotein 330 (megalin) receptor (Moestrup et al., 1993). These receptors are involved in the internalisation of hydrophobic metabolites.

Integrins and the formyl like peptide receptor protein (FLPR) have both been reported to be recruited in uPAR endocytosis, with integrins being associated with uPAR in caveolae (Blasi & Carmeliet, 2002) (Fig. 1.9). However, on the surface of the cells the interaction with integrins, vitronectin, high molecular weight kallikrein and fibronectin is thought to be an adhesive role or one that mediates intracellular signalling events (Andreasen et al., 2000, Liu et al., 2002, Wei et al., 1996, Wei et al., 1999).

It has been suggested that particles up to 200 nm can be endocytosed by a clathrin-dependent pathway, whereas particles between 200 nm and 500 nm rely on caveolae for internalisation in B16-F10 cells (murine melanoma) (Rejman et al., 2004). This fact, in conjunction with the knowledge that uPAR is endocytosed in conjunction with LDLRP in clathrin-coated vesicles and it is transported to the early endosome (Czekay et al., 2001), means that some degree of control over the endocytic pathway may be gained from adjusting ligand-polyplex size to < 200 nm.

Endocytosis, endocytic mechanisms and intracellular trafficking are often studied using fluorescent probes and they can be utilised in live cell imaging experiments. In addition to labelling with fluorescent dyes the use of fluorescent protein as genetically engineered fusion proteins (e.g. green fluorescent protein tagging) has also enabled a greater understanding of intracellular pathways (for a review see Watson et al., 2005).

After endocytosis uPAR has been shown to be recycled to the membrane without degradation in human monocyte-like U937 cells and in murine LB6 clone 19 cells, a mouse cell line transfected to overexpress the human uPAR (Nykjaer et al., 1997). The endocytosis in U937 cells is an interesting case both because this is the most widely studied cell line with regards to uPAR and because there is a fusion between the mixed-lineage leukaemia translocated to 10 gene and the gene coding for phosphatidylinositol-binding clathrin assembly protein (Cottier et al., 2004). This may mean that this pathway is aberrant in this cell line (Cottier et al., 2004). Through a different endocytic mechanism uPAR has been shown to associate with the cation-independent mannose 6-phosphate insulin-like growth factor-II receptor independent of uPA for endocytosis and lysosomal degradation in human fibroblasts (Nykjaer et al., 1998).

1.4.4 Targeting uPAR

Clearly, uPAR presents a new and interesting target that has the potential to promote tumour-specific drug delivery. Indeed, a German company, Wilex, has several small molecule and antibody products aimed at the urokinase system in clinical trials (Willex, 2005). These include a small molecule serine protease inhibitor (WX-UK1) in two Phase 1b clinical trials (Willex, 2005). Willex are also testing an orally available small molecule serine protease inhibitor (WX-671) to block metastasis and tumour growth in Phase I clinical trials (Willex, 2005). Small molecule uPA inhibitors are being developed, as are ligands for soluble uPAR (Willex, 2005).

An alternative approach has been to target uPAR using a diphtheria toxin/urokinase fusion protein (Ramage et al., 2003, Vallera et al., 2002). A recombinant fusion protein of the amino terminal fragment of uPA to diphtheria toxin (DTAT) was made (Vallera et al., 2002). An *in vitro* assay using human glioblastoma cells (U118MG) showed that DTAT toxicity had an $IC_{50} < 1$ nM. *In vivo* DTAT regressed U118MG tumours in mice (Vallera et al., 2002). DTAT toxicity, tested against acute myeloid leukaemia (AML) cells expressing uPAR *in vitro*, correlated with uPAR expression (Ramage et al., 2003). Fusion proteins of ATF with pseudomonas exotoxin have also been made, these were shown to be endocytosed without the α_2 -macroglobulin receptor with cytotoxicity being retained; $IC_{50} = 2.8$ pM in MCF7 cells (Rajagopal & Kreitman, 2000). So far, few studies have used uPAR as a target to improve gene therapy. Some of the advantages/disadvantages of targeting uPAR that have been identified are in Table 1.4.