

In addition to the many specific components of the uPA system shown in Fig. 1.8, internalisation of uPAR is complex. It proceeds after the recruitment of other cell membrane components (Blasi, 1997, Blasi & Carmeliet, 2002, Nykjaer et al., 1998, Nykjaer et al., 1997, Nykjaer et al., 1994a). There appears to be many mechanisms by which uPAR internalisation can occur. An understanding of the endocytic fate is important to the proposed use of uPAR as a target and it is discussed in detail in Section 1.4.3.

1.4.2 Evidence for Over-Expression of uPAR in Cancer

It has been shown that uPAR is over-expressed in a variety of cancers including: monocytic, myelogenous and megakaryocytic leukemias (Lanza et al., 1998, Plesner et al., 1994), bladder transitional carcinomas (Carriero et al., 1997), thyroid (Hudson & McReynolds, 1997), stomach (Plebani et al., 1997), liver (De Petro et al., 1998), pleura (Shetty & Idell, 1998), lung (Morita et al., 1998), pancreatic (Taniguchi et al., 1998), ovarian (Sier et al., 1998) cancers and glioblastomas (Mori et al., 2000). The upregulation of uPAR has also been shown in prostate cancer cell lines by microarray analysis (Han et al., 2002). This group found an increase (2.2x to 10.3x) in several cell lines in comparison with normal pancreas cells (Han et al., 2002). Lanza et al. (1998) found that those patients with acute myeloid leukaemia (M5 French-British-American subtype) having the worst survival rates showed a significant increase in uPAR levels ($>12 \times 10^3$ antibody binding capacity (ABC)/cell compared to $<12 \times 10^3$ ABC/cell) in their tumour (Lanza et al., 1998). It was also shown that high uPAR expression was associated with chromosome abnormalities and disease relapse (Lanza et al., 1998). Similarly, Suzuki et al. (1998) reported that expression of uPAR in colorectal carcinomas correlated with increasing disease severity, whereas there was no detectable expression in matched normal cells.

The number of uPAR receptors expressed per cell has been determined using radioligand binding assays for several cell lines. U937 (human histocytic lymphoma monocyte) cells have ~31000 receptors/cell and MCF-7 (human breast cancer epithelial) cells have ~4600 receptors/cell (Rajagopal & Kreitman, 2000). All prostate cancer cells (Gleason score 4-9) tested by Garilov et al. (2001) were positive for uPAR protein expression. The Gleason score, or grade, is an indicator of how differentiated the tumour cells are. Well differentiated cells (i.e. normal cells) have a score of 1 and poorly differentiated cells have higher scores. Overall it is apparent that in many cancers

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).