Identification of a novel urokinase receptor splice variant and its prognostic relevance in breast cancer.

The cellular receptor for urokinase-type plasminogen activator, uPAR, plays a central role in both cell surface-associated proteolysis and cellular adhesion. In the present study, we systematically searched for splice variants of uPAR mRNA in human cells and tumor tissues by qualitative RT-PCR using specific primers for uPAR exons 1 and 6. Beside the wild-type (wt) uPAR mRNA and the previously described splice variant lacking exon 5 (uPAR-del5), a novel splice variant lacking both exons 4 and 5 (uPAR-del4/5) was found predominantly in various cancer cell lines. To elucidate whether alternatively spliced uPAR mRNA may be translated and post-translationally processed, we generated stably transfected Chinese hamster ovary cells, which harbor expression plasmids of wt uPAR and various uPAR variants including uPAR-del5 and uPAR-del4/5. By ELISA, flow cytofluorometry, and Western blot analysis, we confirmed synthesis and secretion of wt uPAR and the uPAR variants by the use of domain-specific monoclonal antibodies against uPAR. For quantification of uPAR mRNA variants, we established two highly sensitive real-time RT-PCR assays based on LightCycler technology. Study of their expression in a representative set of breast cancer tissues indicated that the novel mRNA variant uPAR-del4/5 was expressed...
very frequently and independently of uPAR mRNA variants covering exon 4 (uPAR-wt and uPAR-del5). Higher uPAR-del4/5 expression was significantly associated with shorter disease-free survival (p = 0.0004) of breast cancer patients. These results suggest that uPAR-del4/5 mRNA may serve as a novel prognostic marker in breast cancer.