Quantitative RT-PCR assays for the determination of urokinase-type plasminogen activator and plasminogen activator inhibitor type 1 mRNA in primary tumor tissue of breast cancer patients: comparison to antigen quantification by ELISA.

Abstract:
Urokinase-type plasminogen activator (uPA) and its inhibitor plasminogen activator inhibitor type 1 (PAI-1) play a key role in tumor-associated processes such as the degradation of extracellular matrix proteins, tissue remodeling, cell adhesion, migration, and invasion. High antigen levels of uPA and PAI-1 in tumor tissue of various solid malignant tumors, including breast cancer, are associated with poor patient prognosis. In the present study, we examined whether analysis of uPA and PAI-1 mRNA expression represents an alternative to the measurement of the respective antigen levels in breast cancer. Highly sensitive quantitative real-time PCR (QPCR) assays, based on the LightCycler technology, were established to quantify uPA and PAI-1 mRNA expression in breast cancer cell lines as well as in tumor tissue of breast cancer patients. mRNA concentrations were normalized to the expression level of the housekeeping gene h-G6PDH. The respective uPA and PAI-1 antigen concentrations were determined by established ELISA formats. QPCR mean interassay variation coefficients were 11% (uPA) and 8% (PAI-1). In breast cancer cell lines, mRNA and antigen values were highly correlated for both
uPA and PAI-1 (each: rs=0.95; p<0.001). In contrast, correlations between uPA/PAI-1 mRNA and protein in the breast cancer samples were found to be distinctly weaker or not significant. Thus, quantitative determination of mRNA expression for both factors does not mirror antigen levels in breast cancer tissue, possibly due to posttranscriptional regulation. Except for nodal status being inversely correlated with uPA mRNA levels, no significant interrelations were observed between uPA or PAI-1 mRNA expression and clinicopathological parameters. On the protein level, elevated uPA and PAI-1 values were associated with a negative steroid hormone receptor status. In conclusion, the implementation of mRNA quantification of uPA and PAI-1 in breast tumors is unable to serve as a one-to-one substitution for antigen determination by ELISA.