It is well known that various proteases are involved in the proteolysis of the extracellular matrix during cancer invasion and metastasis. Among the various proteases, the urokinase-type plasminogen activator (u-PA) system has been well documented in cancer invasion and metastasis. u-PA activates plasminogen to an active form of plasmin, a key enzyme of fibrinolysis. Plasmin activates matrix prometalloproteinases, which are proenzymes, to their active forms, including type IV collagenase, gelatinases, and stromelysins. Plasmin itself also degrades extracellular proteins, which consist of laminin, fibronectin and type IV collagen. Moreover, plasmin converts single chain u-PA (pro-u-PA) to its active form, two chain u-PA. Plasminogen activator inhibitor-1 (PAI-1) inhibits the action of plasminogen activators such as u-PA and tissue PA (t-PA). u-PA, secreted from cancer cells, must bind to a specific receptor (u-PAR) to become active. These findings suggest that the u-PA system plays a key role in cancer invasion and metastasis as shown in Fig 1. It has been suggested that the presence of u-PA, PAI-1, and the u-PAR of the u-PA system in breast cancer tissue is a strong prognostic factor, independent of classical risk factors such as axillary lymph node involvement, tumor size, and estrogen receptor (ER) status.

It has been shown that adjuvant chemo- and/or endocrine therapy can improve disease-free survival in breast cancer. However, more than 80% of Japanese node-negative breast cancer patients do not have recurrence. Those who will not recur would not need adjuvant therapy. Therefore, it is important to determine the risk of recurrence of node-negative breast cancer in order to choose an appropriate adjuvant therapy.

We prospectively studied the antigen levels of the u-PA system in collaboration with three other institutions, the Departments of Surgery at Osaka NTT Hospital, Osaka National Hospital, and Osaka Medical Center for Cancer and Cardiovascular Diseases, to confirm the prognostic value of u-PA, t-PA, and PAI-1 levels in association with other prognostic factors. Here, we review our previously reported studies regarding the u-PA system in breast cancer recurrence.

**u-PA Antigen Level in Primary Breast Cancer**

The amount of u-PA antigen in the cytosol fractions of 226 breast cancer specimens was measured after radical operations, by enzyme-linked
immunoblot assay (ELISA). The median follow-up of the 226 patients was 60 months. Since the median value of u-PA was 0.29 ng/mg protein, the cut-off level was set at 0.3 ng/mg protein to discriminate high and low u-PA antigen levels. The relationships between u-PA levels and clinicopathological factors was investigated. The levels of u-PA in breast cancer tissues did not correlate with the number of involved lymph nodes, hormone receptor status, patient age or type of adjuvant therapy. The only correlation observed was between tumor size and u-PA antigen levels. T3 and T4 tumors had higher u-PA levels compared with T1 tumors.

Table 1 shows the relative risk of disease recurrence for a number of different variables (univariate analysis using the proportional hazards general linear model). Among the various variables, lymph node status and tumor size were the strongest predictors of recurrence. The next strongest predictor was ER status, followed by the u-PA antigen level and PgR-status. The relative risk of recurrence in patients with a higher u-PA antigen was 2.04 compared with patients with a lower u-PA antigen level. The relative risks of disease recurrence for axillary node status, ER-positivity, PgR-positivity and tumor sizes were 2.15, 2.73, 1.83 and 2.63, respectively. Moreover, multivariate analysis of the following parameters was conducted: axillary node status, u-PA antigen level, ER status, type of adjuvant therapy and menopausal status (Table 2). Among these parameters, axillary node status, ER-status and u-PA antigen level were significantly related to recurrence. Therefore, not only axillary node status and ER-positivity, but also u-PA antigen level in the breast cancer cytosol are independent prognostic factors.

On analysis with the Kaplan-Meier method, patients with breast cancer showing high levels of u-PA antigen had a significantly shorter disease-free survival than patients with low levels of u-PA. Using the logrank test, the p-value of the disease-free survival curve between patients with high u-PA levels and low u-PA levels was 0.012, showing a statistically significant difference.

Node-Negative Breast Cancer

We have investigated the levels of u-PA, t-PA, and PAI-1 antigen in breast cancer cytosol fractions and have studied their relationship with disease-free survival during a mean follow-up period of 53 months in 130 patients. All the patients were operated on and followed-up at Osaka University Hospital and three other affiliated hospitals in Osaka, Japan. u-PA, PAI-1, and t-PA antigen levels were measured by an ELISA kit using the cytosol fractions that were prepared for hormone receptor assay. The cut-off points for u-PA, t-PA, and PAI-1 were 0.3 ng/mg protein, 6.0 ng/mg protein, and 1.0 ng/mg protein, respectively. We determined the prognostic value of the u-PA, t-PA, and PAI-1 levels on disease-free survival using univariate and multivariate analysis.

Seventeen of 130 patients developed recurrence during the follow-up period. Patients with either high u-PA or high PAI-1 showed significantly lower disease-free survival rates than those with low u-PA or PAI-1, respectively (P=0.006 and 0.032, respectively) (Fig 2). Patients with low t-PA showed a significantly lower DFS rate than those with high t-PA (P=0.028) (Fig 2).