Higher Expression of Urokinase-type Plasminogen Activator And Plasminogen Activator Inhibitor Type 1 in SGT Cell Line

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Urokinase-type plasminogen activator (uPA) and plasminogen activator type 1 (PAI-1) inhibitor contribute to the invasiveness of many carcinomas. It will be helpful to study clinical behavior of patients with malignant tumor by analysis of their expression. Expression of uPA and PAI-1 in human salivary gland tumors has been rarely reported in vitro. The purpose of this study were to investigate the protein expression of uPA and PAI-1 in SGT cell line compared to oral SCC and HeLa cell lines and to study migration and adhesion assay. All the cell lines were cultured under DMEM with 10% FBS at at 37°C in a 5% CO\textsubscript{2} incubator. We studied a possible association between cytosolic uPA and PA-1 concentrations in SGT cell line compared to any other cell lines through cell migration and adhesion assay, and enzyme-linked immunoassay(ELISA). In migration assay SGT cell line was about 2.5–4 folds higher than another cell lines. In adhesion assay SGT cell line was about 1.1–2 folds higher than another cell lines. uPA cytosolic concentrations of SGT cell line was about 3–10 folds, while PAI-1 was about 2.5–10 folds. Oral SCC cell lines were the lowest value. Both uPA and PAI-1 concentrations were correlated with migration and adhesion assay. High cytosolic concentrations of uPA and PAI-1 was correlated with migration and adhesion assay. It suggested that these markers might be specific marker for SGT cell line and would be contributed to treatment and prognosis of human salivary gland adenocarcinoma.

Key words: UPA, PAI–1 Expression, SGT Cell Line

I. INTRODUCTION

Adenocarcinoma NOS is the third most common ma-
lignant tumor of the salivary glands\textsuperscript{1}. Moreover, because salivary gland adenocarcinoma is poorly represented by cultured cell lines, considerable knowledge is lacking concerning representative treatment drug\textsuperscript{2}. Current therapy does not significantly improve survival rates of this adenocarcinoma. Thus, investigating new therapeutic modalities against adenocarcinoma is necessary. It is important to establish markers selecting patients for adjuvant therapy.

The urokinase (uPA) and plasminogen activator inhibitor (PAI) system is involved in remodelling tissues during invasion and metastasis of tumor cells\textsuperscript{3-5}. PAI–1 is a direct and fast acting inhibitor of urokinase (uPA)\textsuperscript{6-7}. As a consequence conversion of plasminogen to plasmin is reduced, PAI–1 also binds to the extracellular matrix compound vitronectin, thereby limiting the binding of vitronectin to the integrin receptor \( \alpha_v \beta_3 \)\textsuperscript{5,7,8}. Both mechanisms which inhibit proteolysis, adhesion and migration have been demonstrated in vascular smooth muscle cells\textsuperscript{7,9}, macrophages\textsuperscript{10} and mouse embryonic fibroblasts\textsuperscript{6}. However, in tumor cells the situation seems to be complex. It has been shown that PAI–1 can alternatively retard or enhance cell migration and adhesion\textsuperscript{4,9,11,12}, while it has been reported that the concentration of PAI–1 is critical for tumor invasion in vivo\textsuperscript{13}. At physiological concentrations host PAI–1 was reported to promote in vivo tumor invasion and angiogenesis. In contrast, its influence on cell adhesion and migration seems to depend on the cell type, conformation and on the concentration. Concentrations of uPA and PAI–1 in cytosolic fractions of endometrial carcinomas were associated with time to relapse\textsuperscript{13}. However, relatively rarely has been known in salivary gland tumor in vivo and in vitro study\textsuperscript{13}.

The purpose of this study was to investigate the protein expression of urokinase–type plasminogen activator and plasminogen activator inhibitor type in SGT cell line through migration, adhesion assay and ELISA.

II. MATERIALS AND METHODS

1. Culture Condition

SGT, SCC 15, HN 4 and HeLa cell line were cultured under Dulbecco’s modified Eagle’s medium (DMEM, Hyclone USA) with 10% FBS containing penicillin at 37°C in a 5% CO\textsubscript{2} incubator. The growth medium was changed twice weekly. In 70~80% confluency, the cells were treated with 0.05% trypsin and EDTA (Biowittacker, USA) in calcium and magnesium free PBS (pH 7.3). These cells dispersed in a single cell suspension were washed twice by DMEM and then seeded into T-25 ml flask at a density of 10\textsuperscript{4} or 10\textsuperscript{5} cells in 4 ml of growth medium in a 5% CO\textsubscript{2} incubator at 37°C. Subculture was made on 4 or 5 day intervals.

2. Cell Migration Assay

A modified Boyden chamber was used for the cell migration assay, as described previously with slight modifications. The chamber (Falcon) consisted of upper and lower compartments separated by a polyethylene terephthalate track-etched filter (6.4 mm diameter) with 8 mm pores. The lower side of the filter was coated overnight with 10 mg/ml of type IV collagen or BSA and then blocked with 3% BSA for 1h at room temperature. The lower compartment of the chamber was filled with 700 ml of serum free medium containing 0.1% BSA and then blocked with 3% BSA for 1h at 37°C. Subculture was made on 4 or 5 day intervals.

A 200 ml aliquot of suspended cells (1 × 10\textsuperscript{5} cells in serum–free medium containing 0.1% BSA) was placed in the upper compartment of the chamber. After a 12h incubation at 37°C, cells that migrated to the lower side of the filter were quantified by light microscopy under a high power field(×200). For each of the trip-
licate experiments, cells in five randomly chosen fields were counted, and averaged.

3. Cell Adhesion Assay

A standard static adhesion assay was carried out using a 96-well microtiter plate coated with 10μg/ml of type IV collagen or BSA. Cell suspensions (2×10^4 cells/well) were added to the wells and allowed to adhere at 37°C for 60 min. The wells were washed three times with PBS and fixed with methanol for 15 min at room temperature. Attached cells were stained with 0.5% crystal violet and lysed in 2% SDS. The absorbance was measured at 590 nm with a microplate reader.

4. ELISA Analysis

Subsequently, commercially available kits were applied (IMUBIND Tissue uPA ELISA Kit, product no. 894 and IMUBIND Tissue PAI-1 ELISA Kit, product no. 82, American Diagnostica Inc., USA) according to the manufacturer’s instructions. The ELISA employs murine anti-human uPA or PAI-1 as the capture antibodies. Samples were incubated in precoated microtest wells. Detection was achieved with secondary biotinylated antibodies directed against the bound uPA or PAI-1 molecules. Detection was performed by streptavidin conjugated horseradish peroxidase and addition of a perborate/3,3′,3′,5′-tetra- methylbenzidine (TMB) substrate creating a blue colored solution as a consequence of reaction with HRP. uPA and PAI-1 concentrations were measured by determination of absorbances at 450 nm and comparing the values with those of a standard curve.

III. RESULTS

The migration responses of SGT cell lines to type IV collagen, were significantly stronger than those of oral SCC and HeLa cell lines. The present study confirmed the preferential migration of SGT cells to types IV collagen (Fig. 1). The relative migration of cells to type IV collagen was calculated with BSA-induced migration scored as 100% in every cell line used. The results showed that the relative migrations of SGT cell

![Fig. 1](image1.png)

![Fig. 2](image2.png)

![Fig. 3](image3.png)

![Fig. 4](image4.png)
line to types IV collagen were 2.5 to 4 fold those of the SCC 15, HN 4 and HeLa cell line, respectively (Fig. 1). And In adhesion assay SGT cell line was about 1,1–2 folds higher than another cell lines (Fig. 2). Cell adhesion was examined for 1h after plating on ECM substrates, Although the adhesion ability of each cell line to different substrates varied, the adhesion of SGT cells to type IV collagen was relatively higher than that of oral SCC and HeLa cell lines, There was no great difference in the adhesion ability to type IV collagen, between SCC 15, HN 4 and HeLa cell line (Fig. 2). We observed a 3 to 10 folds higher PAI-1 concentration in SGT cell line compared to SCC 15, HN 4 and HeLa cell line (Fig. 4). While uPA cytosolic concentrations of SGT cell line was about 3–10 folds (Fig. 3). There was no great difference in the adhesion ability to type IV collagen, between SCC 15, HN 4 and HeLa cell line (Fig. 3). But it was thought that both uPA and PAI-1 concentrations would be correlated with migration and adhesion assay.

IV. DISCUSSION

It is important to establish markers selecting patients for adjuvant therapy. Due to severe side effects overtreatment should be avoided and the selection process should be as specific as possible. The prognostic relevance of the urokinase activation system has been intensively studied. There are conflicting data regarding the prognostic role of uPA and PAI-1 in cancer, Especially in breast cancer high levels of uPA and PAI-1 have been shown to predict poor prognosis. Endometrial carcinomas with advanced stage and high grade were reported to have higher uPA and PAI-1 concentrations than tumors of less advanced stages. Elevated expression of PAI-1 in ovarian cancer have been reported to be associated with shorter survival. Cytosolic concentrations of both, PAI-1 and uPA were clearly associated with recurrence free survival, but multivariate analysis resulted in a significant association for uPA but not for PAI-1. In this respect uPA and PAI-1 may help to identify patients at risk of relapse. Nevertheless, it should be considered that uPA and PAI-1 are only moderate size prognostic factors.

Higher level expression of uPA and PAI-1 were found in adenoid cystic carcinoma (AdCC) than in normal salivary gland. Cell migration through the ECM is mediated via cell surface integrins that interact with both the ECM and cell surface ligands. The migration responses of all AdCC cell lines to the ECM, especially type IV collagen, were significantly stronger than those of SCC cell lines, while both cell types generally showed similar patterns of integrin subunits. AdCC cells produced a large amount of uPA and degrade ECM via the uPA-plasmin cascade. Combined, these findings suggested that the characteristic properties of AdCC cells (e.g., invasion and metastasis) were mainly regulated by the uPA/uPAR system. In addition to facilitating cell surface-based plasminogen activation, which generated a proteolytic cascade contributing to matrix degradation during tumor invasion in cell adhesion and migration. However, relatively little has been known in salivary gland adenocarcinoma in vivo and in vitro study. Great efforts have not been made to identify factors that predict prognosis in salivary gland adenocarcinomas. Therefore, higher grade carcinomas are more likely to exceed critical concentrations of PAI-1, which will probably contribute to the malignant phenotype of salivary gland adenocarcinoma by similar results in this study. Unfortunately, the cell line number in this study was not sufficient to perform a reliable stat-
istical analysis of interactions between uPA and PAI–1. This analysis suggested that both, high uPA and high PAI–1 may be associated with a particularly bad prognosis in clinical study. Interestingly, the combination of high uPA and PAI–1 concentrations in tumors may reveal an increased risk of cancer progression. It would be interesting to report that a combination of PAI–1 and uPA allows an even better identification of high risk malignancy than only one factor. However, for statistical reasons we could not analyze interactions of these factors, due to the relatively small number of cell line. Thus, this observation requires conformation in a larger cohort in future study.

In conclusion, we have identified higher uPA and PAI–1 expression as specific markers for SGT cell line than any other cell lines. The combination of high cytosolic concentrations of uPA and high PAI–1 may reveal a reliable marker with increased risk of salivary gland adenocarcinoma progression.

V. REFERENCES

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