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In vitro invasion of transfected human breast epithelial cells MCF10A-neoT

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Introduction

Tumor invasion and metastasis are responsible for the progression of malignant disease. These processes are facilitated by the upregulation of various types of proteinases. In recent years, experimental and clinical studies have been carried out using inhibitors of MMPs,[1,2] plasmin and plasminogen activators,[3] as well as cysteine proteinases.[4,5,6,7] They inhibit either growth,[7] motility[8] or the invasive potential[4,5] of various types of tumours. The aim of the study was to evaluate the effectiveness of synthetic and peptide proteinase inhibitors in reducing *in vitro* invasion of MCF10A cells, transfected with ras oncogene.

Material and methods

MCF10A-neoT cell line was established by transfection of MCF10A cells with T24 c-Ha-ras oncogene[9] and has an acquired ability to grow as xenograft in nude mice, forming small nodules, which progress sporadically into invasive carcinomas of different histological types. Cells were grown to 80 % confluency and 24 h before harvesting, the medium was replaced with SFM (serum free medium).

Invasion assay

The method described by Albini and co-workers[10] was used with minor modifications. Polycarbonate filters (Costar, USA) with 12 μm porosity were coated with fibronectin (25 ng/mm, Sigma, Germany) on the lower surface and with Matrigel (0.9 μg/mm, Becton Dickinson, USA) on the upper surface, dried overnight and reconstituted with 200 μl SFM for 1 h at 37 °C. Maximum inhibition of invasion was observed using SFM containing proteinase inhibitors (Bachem, Switzerland) at the following non-cytotoxic concentrations: 10 μM E64, 100 μM E64-d, 20 μM Ca-074, 0.5 μM Ca-074Me, 20 μM Z-FA-FMK, 0.5 μM Clik 148, 50 μM Z-FF-CHN_2, 1 μM pepstatin A, 100 μg/ml aprotinin and 10 μM BB94. Cytotoxicity was determined using MTT (1-(4,5 dimethyltiazol-2-yl)-2,5 diphenyl tetrazolium bromide) viability assay. Cells were harvested and seeded (200,000 cells in 0.5 ml SFM) to the upper chamber. After 21 h incubation, MTT (Sigma, Germany) at the final concentration of 0.5 mg/ml was added.[3] The cells were further incubated for 3 h at 37 °C to allow the formation of formazan crystals. The crystals from upper and lower chambers were separated and pelleted by centrifugation at 15000 rpm for 5 min and dissolved in 1 ml of DMSO. Optical density (OD) was measured at 570 nm (reference filter 690 nm). Invasiveness of the cells was calculated as the ratio of the OD in the lower chamber to the sum of ODs in the lower and upper chambers.

Results

The ability of inhibitors of cysteine, aspartic, serine and metalloproteinases to reduce inva-

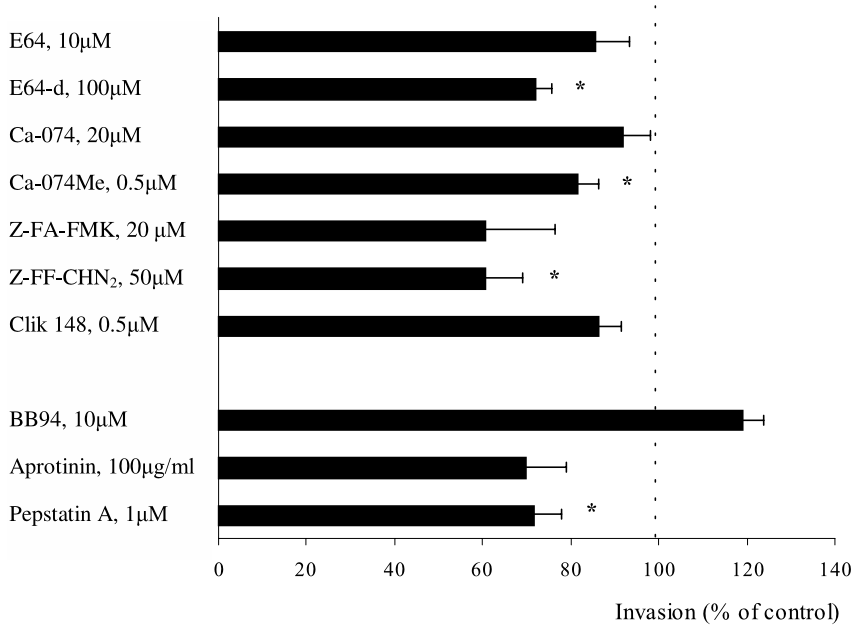

Figure 1. Inhibition of MCF10A-neoT cells by synthetic inhibitors of invasion.

sion of MCF10A-neoT cells was determined (Figure 1). Membrane-permeant cysteine proteinase inhibitors, E64-d (28 %) and Z-FF-CHN_2 (40 %), were the most effective, followed by the selective CatB inhibitor, Ca-074Me (about 20 %). Inhibition of invasion by Z-FA-FMK (40 %) and the selective CatL inhibitor, Clik 148 (14 %) was not statistically significant. Among the inhibitors of other types of proteinases, only pepstatin significantly inhibited the invasion (28 %). Aprotinin reduced the invasiveness to the similar extent. The broad spectrum matrix metalloproteinase inhibitor BB94 did not inhibit invasiveness.

Data is expressed as percentage of the control. Error bars depict standard error of the mean values of three independent experiments. Statistical significance (*) was determined by two tailed t-test with assumed equal variance, and $p < 0.05$ was considered significant.

Discussion

The MCF10A-neoT cell line was used to determine the effect of proteinase inhibitors on invasion. Cysteine proteinase inhibitors were found to be more effective than the inhibitors of other classes of proteinases. Therefore, we conclude that cysteine proteinases contribute significantly to the process of invasion. CatB inhibitors proved more effective than CatL inhibitors, suggesting that CatB plays a more important role than CatL. Since the derivatives of CatB inhibitors, which can enter the cells, were found to be most effective, the cells probably degrade collagen also intracellularly, as reported previously. The aspartic proteinase inhibitor pepstatin A also reduced the invasion, so CatD may also be involved in breast tumor cell invasion.[12] Similar to reports in human esophageal and ovarian carcinoma cells *in vivo*[13], we did not observe inhibition of invasion by the broad spectrum inhibitor of MMPs, BB94. This is in contrast to the previous reports of its inhibitory effect

if invasion in other cell types.[4,5] Such discrepancies may reflect differences in the expression and activation of MMPs in various cells. Our results support the hypothesis that the cysteine proteinase CatB plays an active role in invasion of transformed human breast cell lines. These findings could have an impact on the search for new anti-invasive and anti-metastatic agents

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