

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

Nataša Sever, Nataša Levičar, Irena Zajc, Aleš Bervar and Tamara T. Lah

Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Večna pot 111, 1000 Ljubljana, Slovenia

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 motility⁸ 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 oncogene⁹ 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-workers¹⁰ was used with minor modifications. Polycarbonate filters (Costar, USA) with 12 µm po-

rosity 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 Clk 148, 50 µM Z-FF-CHN₂, 1 µM pepstatin A, 100 µg/ml aprotinin and 10 µM BB94. Cytotoxicity was determined using MTT (1-(4,5 dimethylthiazol-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-

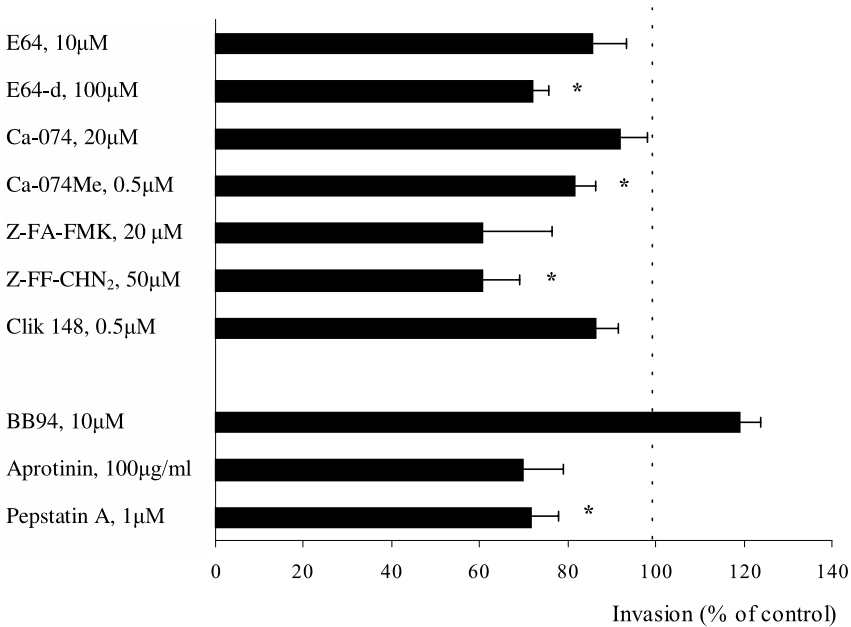


Figure 1. Inhibition of MCF10A-neoT cells by synthetic inhibitors of invasion.

sion of MCF10A-neoT cells was determined (Figure 1). Membrane-permeant cysteine proteinase inhibitors, E64-d (28%) and Z-FF-CHN₂ (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

Acknowledgements

The authors thank Dr.B.F. Sloane (Wayne State University, USA) for the cells, Dr. P. Brown (British Biotech Pharmaceutical,UK) for the inhibitor BB94, Dr. N. Katunuma (Tookushima Bunri University, Japan) for Clk 148 and Dr. C.J. Van Noorden (University of Amsterdam, The Netherlands) for the inhibitor Z-FA-FMK. This work was supported by the Ministry of Education, Science and Sport, programme #105-509).

References

1. Kohn CE, Liotta LA. Molecular insights into cancer invasion: Strategies for prevention and intervention. *Cancer Res* 1995; **55**: 1856-62.
2. Botos I, Scapozza L, Zhang D, Liotta LA, Meyer EF. Batimastat, a potent matrix metalloproteinase inhibitor, exhibits an unexpected mode of binding. *Proc Natl Acad Sci USA* 1996; **93**: 2749-54.
3. Holst-Hansen C, Johannessen B, Hoyer-Hansen G, Romer J, Ellis V, Br nner N. Urokinase-type plasminogen activation in three human breast cancer cell lines correlates with their *in vitro* invasiveness. *Clin Exp Metastas* 1996; **14**: 279-307.
4. Kolkhorst V, St rzenbecher J, Wiederanders B. Inhibition of tumor cell invasion by proteinase inhibitors: correlation with the proteinase profile. *J Cancer Res Clin Oncol* 1998; **124**: 598-606.
5. Stonelake PS, Jones CE, Neoptolomos JP, Baker PR. Proteinase inhibitors reduce basement membrane degradation by human breast cancer cell line. *Br J Cancer* 1997; **75**: 951-9.
6. Bjornland K, Buo L, Kjoniksen I, Larsen M, Fodstad O, Johansen HT, Aasen AO. Cysteine proteinase inhibitors reduce malignant melanoma cell invasion *in vitro*. *Anticancer Res* 1996; **16**: 1627-32.
7. Van Noorden CJF, Jonges TGN, Meade Tollin LC, Smith RE, Koehler A. *In vivo* inhibition of cysteine proteinases delays the onset of growth of human pancreatic cancer explants. *Br J Cancer* 2000; **82**: 931-6.
8. Boike G, Lah T, Sloane BF, Rozhin J, Honn K, Guirguis R, Strache ML, Liotta LA, Schiffmann E A possible role for cysteine proteinase and its inhibitors in motility of malignant melanoma and other tumour cells. *Melanoma Res* 1991; **1**: 333-40.
9. Basolo F, Elliot J, Tait L, Chen XQ, Maloney T, Russo IH, Pauley R, Momiki S, Kaamano J, Klein-Szanto AJP, Kozsalka M, Rosso J. Transformation of human breast epithelial cells by c-Ha-ras oncogene. *Mol Carcinogen* 1991; **4**: 25-35.
10. Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aaronson SA, Kozlowski JM, McEwan RN. A rapid *in vitro* assay for quantitation of the invasive potential of tumor cells. *Cancer Res* 1987; **47**: 3239-45.
11. Sameni M, Moin K, Sloane BF. Imaging proteolysis by living human breast cancer cells. *Neoplasia* 2000; **2**: 496-504.
12. Rochefort H, Garcia M, Glondu M, Laurent V, Liaudet E, Rey JM, Roger P. Cathepsin D in breast cancer: Mechanisms and clinical application, a 1999 overview. *Clin Chem Acta* 2000; **291**: 157-70.
13. Della Porta PD, Soeltl R, Krell WH, Collins K, Odonoghue M, Schmitt M, Krueger A. Combined treatment with serine proteinase inhibitor aprotinin and matrix metalloproteinase inhibitor batimastat (BB94) does not prevent invasion of human esophageal and ovarian carcinoma cells *in vivo*. *Anticancer Res* 1999; **19**: 3809-16.