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The effect of E-64 and monoclonal antibodies on proliferative and invasive activity of ras transformed human breast epithelial cell line MCF10A neoT tested in *in vitro* assays

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Introduction

The characteristic of malignant tumours is their potential to invade normal host tissue and to metastasize to distant organs.[1,2] Tumour cell invasion of the extracellular matrix (ECM) is described[3] as a multi-step process. First, tumour cells need to be attached to the components of the ECM. Next step is the local degradation of ECM components, followed by the migration of the tumour cell through locally modified matrix. Invasive behaviour of tumour cells requires proteolytic activity associated with the concerted action of various intra and/or extracellular proteinases, including a cysteine proteinase cathepsin B. Cathepsin B was found localized on the surface of different brain and breast cancer cells, including MCF10A neoT.[4] Another prerequisite for tumour cells to form distant metastases is tumour cell proliferation.[2]

The effect of E-64, an irreversible cysteine proteinase inhibitor and various monoclonal antibodies raised to human cathepsin B, was tested on ras transformed human breast epithelial cell line MCF10A neoT, using *in vitro* proliferation and invasion assays.

Materials and methods

Hybridoma technique, first demonstrated by Köhler and Millstein in 1975[5], was used to prepare mouse anti-cathepsin B monoclonal antibodies. Recombinant cathepsin B was used for immunization of Balb/c mice. For *in vitro* proliferation and invasion assays we used MAbs, derived from two clones of hybridoma cells.

The MCF10A neo T, ras transformed human breast epithelial cell line was used in the in vitro assays. The cells were cultured in DMEM/F12 (1:1), with 5 % foetal calf serum (FCS) and supplemented with insulin, epidermal growth factor, hydrocortisone and antibiotics at 37 °C and 5 % CO_2 in humidified atmosphere, to about 70-80 % confluency. The assays were performed using the same DMEM/F12 medium, however, FCS used was purified on the affinity chromatography column using immobilized papain.

The MTT-cell proliferation assay[6] was used to assess possible influence of inhibitor and antibodies on tumour cell proliferation. It is a quantitative colorimetric assay based on cleavage of the tetrazolium salt MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) into water insoluble, dark-blue formazan crystals by the mitochondrial enzyme succinate-dehydrogenase.[7] Assays were performed in quadruplicates.

To study the effects of MAbs and E-64 on tumour cell invasion, Costar Transwells with 12 mm polycarbonate filters and 12 μm pore size were used. Filters were coated with Matrigel (Becton Dickinson).[8] Assays were performed in triplicates.

Results and conclusions

In the proliferation assay, MAbs derived from clone A and E-64 didn't show any effect in the concentration range tested (2-0,01 μM and 100-0,1 μM). MAbs derived from clone B expressed a slight anti-proliferative effect in a dose-dependent manner (2-0,01 μM).

Results of in vitro invasion assay show a strong inhibitory effect expressed on the invasion of MCF10A neoT cells by E-64. MAbs derived from clone B had no effect, whereas MAbs from clone A expressed a 20 % inhibition compared to control at 1 μM concentration.

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