EVIDENCE OF ANTIANGIOGENIC AND ANTIMETASTATIC ACTIVITIES OF THE RECOMBINANT DISINTEGRIN DOMAIN OF METARGIDIN

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Introduction: Metargidin (metalloprotease-RGD-disintegrin protein) is a transmembrane adamalysin expressed at a higher level by activated endothelial cells. Furthermore, metargidin is the only known adamalysin that possesses a RGD motif at the tip of its disintegrin loop (CRPTRGDCD), which binds integrins αvβ3 and α5β1 (1-3).

Methods: Recombinant human disintegrin domain (RDD) was produced in Escherichia coli, and the effect of purified RDD on different steps of angiogenesis was evaluated in vitro. For in vivo experiments, the RDD gene was inserted into the tetracycline inducible pBi vector. We injected 20 µg each of empty pBi or pBi–RDD, together with 10 µg of the Tet-tTS and 20 µg of the Tet-On plasmids, in sterile 0.9% NaCl into both tibialis cranialis muscles, and electrotransfer was conducted. Eight transcutaneous square electric pulses (200V/cm) were applied for 20 ms by use of two plate electrodes placed apart on the leg at a frequency of 1 Hz by use of a PS-15 electropulsator (Jouan).

Results: At concentrations of 2-10 µg/ml, RDD exhibited inhibitory activities in a variety of in vitro functional assays, including endothelial cell proliferation and adhesion on the integrin substrates, fibronectin, vitronectin or fibrinogen. RDD (10 µg/ml) totally abrogated endothelial cell migration and blocked most capillary formation in a tridimensional fibrin gel. We then examined the effect of skeletal muscle-secreted RDD on the growth of established MDA-MB-231 breast tumors. Mice received plasmids by electrotransfer before being inoculated s.c. with MDA-MB-231 cells. When tumors reached 18 mm³, RDD expression was turned on by doxycycline. Tumor growth in the RDD-treated group was significantly inhibited by 78% as compared with the controls (Fig. 1A). This antitumoral effect was associated with a strong inhibition of tumor angiogenesis (Fig. 1B-C).
Fig. 2: RDD inhibition of MDA-MB-231 tumor growth and angiogenesis. Photographs show control (B) and RDD-treated (C) tumors.

The same gene delivery system was used to investigate the effect of RDD on the B16F10 model of lung metastasis formation. The plasmids were electrophoretically transferred into syngeneic C57BL/6 mice, and doxycycline induction was started immediately. Three days later, these mice received an i.v. injection of B16F10 cells. One week after inoculation, mice were sacrificed, and lung metastases were counted. In the presence of RDD, 74.2% fewer metastatic nodules were detected in the experimental group than the controls (Fig. 2).

Discussion:

Taken together, these results identified this RDD as a potent intrinsic inhibitor of angiogenesis, tumor growth and metastasis, making it a promising tool for use in anticancer treatment.

References: