A DNA VACCINE TARGETING THE ANGIOSTATIN RECEPTOR ANGIOMOTIN INHIBITS ANGIOGENESIS AND SUPPRESSES TUMOR GROWTH

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Introduction: Endogenous angiogenesis inhibitors have shown promise in pre-clinical trials but clinical use has been hampered by low half-life in circulation and high production costs. Here we describe a novel strategy that targets the angiostatin receptor angiomotin by DNA vaccination. In order to design alternative molecules more suited for anti-angiogenic therapy, it is of importance to identify the receptors that mediate the anti-angiogenic effect. We have previously reported the cloning and characterization of the angiostatin receptor angiomotin that is expressed in tumor- and placental endothelium (1). Angiomotin is a membrane associated protein that mediates angiostatin inhibition of endothelial migration and tube formation in vitro. A role of angiomotin in cell motility is also indicated by the finding that angiomotin deficient mouse embryos exhibit a migratory defect in the anterior visceral endoderm at embryonic day 7.5 (2). The functional role of angiomotin as an angiostatin receptor and its expression in angiogenic vessels makes it a possible target for anti-angiogenic therapy.

Method: In order to break tolerance against angiomotin and consequently activate an angiomotin-specific immune response, we vaccinated BALB/c mice by intramuscular injection followed by electroporation with pcDNA3-Amot or empty vector as a negative control as previously described (3). The use of a human angiomotin DNA construct was motivated by the ability of xenogeneic proteins to break immunological tolerance against self antigens. The mice were vaccinated twice with a two weeks interval.

Results: The vaccination procedure generated antibodies that detected angiomotin on the endothelial cell surface. Purified Ig bound to the endothelial cell membrane and inhibited endothelial cell migration. In vivo, DNA vaccination blocked angiogenesis in the matrigel plug assay and prevented growth of transplanted TUBO breast cancer tumors for up to 150 days. The efficacy of angiomotin DNA vaccination as a single agent or in combination with Her2/neu DNA vaccination was tested in the BALB-neuT mammary carcinoma model. A combination of Her2 DNA vaccination with angiomotin DNA vaccination administered in Balb neu T mice at 10 and 12 weeks post birth was able to inhibit tumor protection for over 70 weeks. No toxicity or impairment of normal blood vessels could be detected.

Discussion: We report that DNA vaccination targeting the angiostatin receptor angiomotin mimics the effect of angiostatin and inhibits angiogenesis and tumor growth in mice. These findings provide the rationale to generate antibodies that mimic the effect of angiostatin. This would potentially be of marked advantage to using angiostatin as immunoglobulins have a half-life of 20 days while the half-life of angiostatin is only 3 hours.