Three-Dimensional Imaging of Tumor Angiogenesis in Estrogen-induced Pituitary Prolactinoma and its Inhibitory Effect by Anti-VEGF antibody in Fischer 344 Rats

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BACKGROUND: Estrogen-induced prolactinomas (PRLomas) in Fischer 344 (F344) rats have demonstrated a high level of vascular endothelial growth factor (VEGF) expression associated with marked angiogenesis and angiectasis. Anti-VEGF antibody (bevacizumab, Genentech Inc.) is known to be as an antibody for therapeutic agents designed to inhibit tumor angiogenesis in human cancers. The aim of this study is to elucidate the inhibitory effects of anti-VEGF antibody (Anti-human and murine VEGF mab; G6-31, kindly supplied by Genentech Inc.) on tumor angiogenesis in the rat PRLomas induced by estrogen.

DESIGN: Female F344 rats of 7 weeks of age were injected (i.m.) with 0 (Control), 3000 μg/kg/week of estradiol (E2-treated) for 10 weeks, and the half of E2-treated rats received 5 mg/kg of anti-VEGF antibody (G6-31) intravenously 2 days after each E2 injection for 3 weeks (G6-31-treated). The animals were sacrificed after 13-week of E2 dosage, their pituitary glands were removed and examined histopathologically. The remaining rats in each group (n=6) were examined by three-dimensional (3D) imaging analysis by confocal laser scanning microscopy (CLSM, LSM510-Meta, Carl Zeiss Jena Germany) for (1) microvessels infused by FITC-conjugated gelatin, (2) 3D microvasculature volume, (3) surface area and (4) segmented pattern of the blood vessels detected automatically using IMARIS-Filament tracer software (Carl Zeiss) from 3D fluorescent images.

RESULTS: Histopathological examination revealed that the number of distended blood vessels in the anterior lobe was diminished by G6-31 treatment compare to E2-treated group. In 3D image analysis, the increase of vascular volume and the decrease of surface area were detected in E2-treated group. Furthermore, increase in the number of segmented vessels and shortening of the length of segmented-vessels were observed. The processes of repair were clearly observed in the G6-31 treated group.

CONCLUSION: The segmented pattern and geometry of pituitary vessels of the tumor angiogenesis were clearly elucidated in the pituitary glands stimulated by E2 treatment and treated by anti-VEGF antibody. Thus these 3D image analyses is expected to serve as a useful technique to evaluate the therapeutic effects of anti-angiogenic agent on vascular-rich endocrine tumors.