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## An EGF motif of Del1 suppresses notch function and inhibits efficient angiogenesis *in vivo*

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**Introduction:** Del1, an ECM protein, is known to show pro-angiogenic or anti-angiogenic activities depending on the experimental conditions. It consists of two discoidin domains (C1, C2) and three epidermal growth factor (EGF) motifs (E1, E2, E3), of which E2 has been reported to contain an RGD sequence that binds to integrin receptors and supports endothelial cell survival. In the present study, we provide evidence that an E3C1 fragment suppresses Notch signaling and angiogenesis in explanted tumor model.

**Materials & Methods:** Cells of the human oral squamous cell carcinoma cell line, SCCKN, were injected into nude mice to generate explanted tumors. cDNAs encoding, the E3 and C1 domains of Del1 (E3C1), were inserted into pcDNA3D (pE3C1) and injected into the tumors every 7 days with a transfection reagent, jet-PEI. Tumor angiogenesis was evaluated by immunohistochemistry with antibodies for PECAM, von Willebrand factor and PDGF-beta and intravenous injection of *Lycopersicon esculentum* lectin. The signal transduction of notch was analyzed by western blotting.

**Results:** Treatment with pE3C1 suppressed the growth of explanted tumors and improved life prognosis of mice. In localization with immunostaining of PECAM or von Willebrand factor, and angiography with *Lycopersicon esculentum* lectin, vasculature without lumen was increased by gene therapy of Del1 fragment. Among the treatment with pE3C1 and control, the PDGF beta staining cells, which is marker of tip cells in angiogenesis, were increased. Western blotting of human umbilical endothelial cells cultured with an E3C1 recombinant protein showed the decreased expression of active notch and hey1.

**Discussion:** The phenotype of tumor vascular by treated with pE3C1 was analogous to the phenotype of inhibited of notch. In our study, it is suggested that the effective angiogenesis was inhibited by E3C1 inhibition of notch function. There is an anti-angiogenic domain, E3C1, adjacent to the RGD in E2 of Del1. The presence of domains with opposing activities next to one another in the same protein could account for the seemingly ambiguous characteristics of Del1 activity on angiogenesis. The EGF domain of Del1 has amino acid sequence, CXDXXXXYXCXC, which was shared by notch and delta. Signaling way of the motif should be investigated for notch function.

### Biography

Kazuaki Nakane has obtained his PhD at Kanazawa University. He is a guest Associate Professor and a specially appointed Researcher in Osaka University. By using an idea of homology, he has developed a new image analysis method and applied this method to detect cancer lesion. He has also been successful in image analysis of complex images which seem to have mathematical structures.

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