

國立交通大學

生物科技研究所

碩士論文

血管內皮生長因子之受體結合部位與人類免疫球蛋白 G1 之 Fc 片  
段之新型融合蛋白降低血管的新生

A novel fusion protein of the receptor binding domain of VEGF and  
human IgG1 Fc portion reduces angiogenesis

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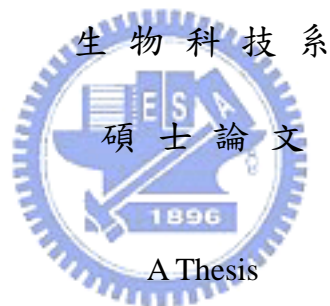
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# 血管內皮生長因子之受體結合部位與人類免疫球蛋白 G1 之 Fc 片段的新型 融合蛋白降低血管的新生

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## 中文摘要

血管新生為在體內由原先已存在血管的基礎上衍生形成出新生血管的生理過程，有別於從原位未分化的血管內皮前期細胞分化到內皮細胞。實質固態的腫瘤細胞生長可藉由血管內皮生長因子激活血管的形成。第一型及第二型的血管內皮生長因子受體已被指出，主要表現在正在增生的血管內皮細胞上。因此，阻礙這兩型受體的正常功能對於治療癌症及一些血管新生依賴的疾病為一具有希望的策略。在此論文研究中，我們設計一個結合血管內皮生長因子之受體結合部位（胺基酸序列 8 到 109）與具有良好免疫特性的人類免疫球蛋白 G1 之 Fc 片段的新穎融合蛋白。RBDV-IgG1 Fc 嵌合式基因被選殖到腺相關病毒表現系統上，作為表現融合蛋白的媒介。我們也呈現一種於人類腎臟上皮細胞 (HEK-293) 的蛋白質表現系統，此法具有高產率及高純度的優勢。藉由酵素結合免疫特性分析與流式細胞儀方法檢試 RBDV-IgG1 Fc 融合蛋白具有與受體結合的活性。並且，由血管內皮細胞生長因子誘導的人類臍帶靜脈內皮細胞的增生也被此融合蛋白所抑制，這說明了 RBDV-IgG1 Fc 在血管內皮細胞生長因子與其受體結合而引導的訊息傳導途徑所扮演的拮抗作用。由這些結果顯示，RBDV-IgG1 Fc 融合蛋白在治療血管新生層面的疾病具有發展的潛力。

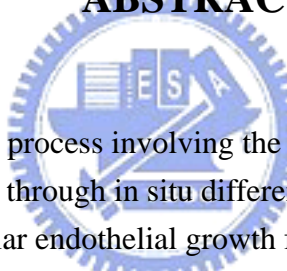
## **A novel fusion protein of the receptor binding domain of VEGF and human IgG1 Fc portion reduces angiogenesis**

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### **ABSTRACT**



Angiogenesis is a physiological process involving the growth of new blood vessels from pre-existing vessels, rather than through in situ differentiation of undifferentiated precursor cells to endothelial cells. Vascular endothelial growth factor (VEGF) is an angiogenic factor that promotes the growth of solid tumor by inducing angiogenesis. The two VEGF receptors, VEGFR-1 and VEGFR-2, have been shown to be expressed preferentially in the proliferating endothelial cells. Thus, inhibiting these two VEGF receptors may be a promising strategy for treatment of cancer and other angiogenesis-dependent diseases. In this study, we designed a novel recombinant fusion protein composed of a targeting domain and an effector domain. The targeting domain is the receptor binding domain of human VEGF (residues 8-109) and the effector domain is the Fc region of a human IgG1 immunoglobulin that can induce a cytolytic immune response against the targeted cells. The chimeric gene, RBDV-IgG1 Fc, was subcloned into AAV expression vector to produce the fusion protein. We also present an approach for the purification of the fusion protein with high yield and high purity from HEK-293 expression system. The binding of RBDV-IgG1 Fc fusion protein to the VEGF receptors was examined by ELISA and flow cytometry. Furthermore, the proliferation of VEGF-induced human umbilical vein endothelial cells (HUVECs) was inhibited by the RBDV-IgG1 Fc, suggesting an antagonistic role in VEGF/VEGF receptors signal pathway. These results showed that RBDV-IgG1 Fc fusion protein is potential for the suppression of angiogenesis in vivo.

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## List of abbreviations

ADCC	Antibody dependent cell-mediated cytotoxicity
AMD	Age-related macular degeneration
aFGF	Acidic fibroblast growth factor
bFGF	Basic fibroblast growth factor
CDC	Cell dependent cytotoxicity
Cys	Cysteine
GIST	Gastro-intestinal stromal tumor
Flt-1	Fms-like tyrosine kinase
HER-2/neu	Human epidermal growth factor receptor 2/neu
HIF-1	Hypoxia inducible factor-1
IFN- $\alpha$	Interferon alpha
IL-6	Interleukin 6
kDa	Kilo dalton
KDR	Kinase domain receptor
NK	Natural killer cell
NO	Nitric oxide
NP	Neuropilin
PDGF	Platelet-derived growth factor
PDGFR- $\beta$	Platelet-derived growth factor receptor-beta
PIGF	Placental growth factor
PI3 kinase	Phosphatidylinositol kinase 3 kinase
RTK	Receptor tyrosine kinase
sFlt-1	Soluble fms-like tyrosine kinase-1
TGF- $\alpha$	Transforming growth factor-alpha
TGF- $\beta$	Transforming growth factor-beta
TNF- $\alpha$	Tumor necrosis factor-alpha
VEGF	Vascular endothelial growth factor
VEGFR	VEGF receptor