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博士の専攻分野の名称	博士（医学）
学位記番号	第 24408 号
学位授与年月日	平成23年3月25日
学位授与の要件	学位規則第4条第1項該当 医学系研究科内科系臨床医学専攻
学位論文名	Angiopoietin-1/Tie2 signal augments basal Notch signal controlling vascular quiescence by inducing delta-like 4 expression through AKT-mediated activation of β-catenin （Angiopoetin1/Tie2シグナルは、AKT経路によるβ-cateninの活性化を介してdelta-like 4の発現を誘導し、血管安定化に重要なNotchシグナルを増強する）
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論文内容の要旨

〔 目 的 〕

Angiopoientin-1 (Angl) regulates both vascular quiescence and angiogenesis through the endothelium-specific receptor tyrosine kinase Tie-2. Angl and Tie2 form distinct signaling complexes at cell-cell and cell-extracellular matrix contacts, thereby playing these distinct roles. In the presence of cell-cell junctions, Angl induces formation of *trans*-associated Tie2, leading to the preferential activation of phosphoinositide 3-kinase (PI3K)/Akt pathway. *Trans*-associated Tie2 also up-regulates Notch ligand delta-like 4 (Dll4). Dll4/Notch signal is known to restrict sprouting angiogenesis and promote vascular quiescence. Therefore, we investigated the mechanism how Angl/Tie2 signal induces Dll4 expression and the role of Dll4/Notch signal in Angl/Tie2 signal-mediated vascular quiescence.

〔 方法ならびに成績 〕

1. Dll4/Notch signaling is promoted by *trans*-associated Tie2

To confirm whether the Dll4 expression is increased by Angl in the presence of cell-cell contacts, human umbilical vein endothelial cells (HUVECs) were stimulated with COMP-Angl (C-Angl), a potent Angl variant, under either confluent or sparse culture condition. By performing real-time reverse transcription-PCR and western blot analyses, I found that Dll4 expression is higher in the confluent HUVECs than in the sparse cells, indicating the basal Notch signal is present in the HUVECs with cell-cell contacts. C-Angl further induced Dll4 expression and subsequent production of Notch intracellular domain (NICD) only in the presence of cell-cell contacts. Depletion of Dll4 by siRNA blocked increase in NICD by C-Angl. These findings indicate that *trans*-associated Tie2 bridged by Angl enhances Notch signaling through upregulation of Dll4.

2. The mechanism by which Ang1/tie2 signal induces Dll4 expression

Since *trans*-associated Tie2 preferentially stimulates PI3K/AKT pathway, we next investigated the involvement of PI3K/AKT pathway in Ang1-induced Dll4 expression. Specific inhibitors for PI3K and AKT prevented C-Ang1-induced Dll4 expression, indicating the requirement of PI3K/AKT signal for Ang1-induced Dll4 expression. Glycogen synthase kinase 3β (GSK3β) is a downstream target of PI3K/AKT pathway, and induces degradation of transcription factor β-catenin (β-cat). Therefore, I investigated whether β-cat is involved in Ang1/Tie2 signal-induced Dll4 expression. GSK3β inhibitors induced Dll4 expression and NICD production. C-Ang1 significantly induced the transcriptional activity of β-cat. Furthermore, depletion of β-cat by siRNA blocked either C-Ang1- or GSK3β inhibitor-induced Dll4 expression. Collectively, these findings indicate that Ang1 stimulates β-cat-dependent transcriptional activity through AKT-mediated inhibition of GSK3β, thereby inducing Dll4 expression.

Evidence that Dll4 expression by the GSK3β inhibitor only occurs in the confluent cells implies the requirement of cell-cell contact-dependent signal in β-cat-mediated Dll4 expression. Consistently, I revealed that inhibition of Notch signal by DAPT, a γ-secertase inhibitor, prevents either C-Ang1- or GSK3β inhibitor-induced Dll4 expression, suggesting the role of cell-cell contact dependent Notch signal in β-cat-mediated Dll4 expression. By performing luciferase reporter assay, I also found that Ang1 and NICD upregulate Dll4 through the RBP-J binding site within the intron 3 of *DLL4 gene* and that β-cat potentiates NICD-induced Dll4 expression. Chromatin immunoprecipitation assay further revealed that β-cat forms a complex with NICD/RBP-J on the Dll4 intron 3 upon stimulation with C-Ang1. Collectively, these findings indicate that Ang1/Tie2 signal recruits β-cat to the NICD/RBP-J complexes on the enhancer region of Dll4 intron 3, thereby upregulating Dll4.

3. The biological significance of Ang1-induced Dll4 expression.

Both Ang1/Tie2 and Dll4/Notch signaling are known to induce formation of vascular basement membrane, a hallmark of vascular maturation and stabilization. Thus, I investigated whether Ang1 induces deposition of collagen type IV, a major basement membrane component, during endothelial cell tube formation in 3D collagen matrices. Extracellular deposition of collagen type IV was evoked by stimulation with C-Ang1. Either inhibition of Notch signal by DAPT or depletion of Dll4 by siRNA blocked C-Ang1-induced deposition of collagen type IV. These findings suggest that Ang1 induces basement membrane formation through the Dll4/Notch signal.

〔 総　　括 〕

In the present study, I investigated the mechanism by which Ang1/Tie2 signal induces Dll4 expression and its role in Ang1/Tie2-induced vascular quiescence. Formation of cell-cell contacts results in activation of Notch signal, which induces Dll4 expression through the RBP-J binding site in the Dll4 intron 3. *Trans*-associated Tie2 bridged by Ang1 activates β-cat through PI3K/AKT signal-mediated inhibition of GSK3β. Stabilized β-cat enhances Notch signal-mediated Dll4 expression by forming a complex with NICD/RBP-J on the Dll4 intron 3, thereby augmenting the Notch signaling. Dll4/Notch signal induced by *trans*-associated Tie2 promotes vascular stabilization through formation of vascular basement membrane.

論文審査の結果の要旨

Angiopoietin (Ang1) は、血管内皮細胞に発現するTie2受容体を介して、血管構造の安定化と血管新生の相反する作用を制御している。張江暉さんはこの理論を背景とし、Ang1/Tie2シグナルは血管の安定化を増強するメカニズムを調べた。DNAマイクロアレイによる解析から、細胞間接着存在下でのみAng1刺激によってNotchシグナルのリガンドであるDll4が上昇していた。Dll4/Notchシグナルは血管安定化に重要である。そこでDll4がAng1による血管安定化作用に関与しているという仮説を立てた。Ang1/Tie2シグナルはPI3K/AKT経路を介してβ-catenin(βCat)を活性化する。活性化されたβCatはDll4/Notchシグナル依存的にDll4の発現を誘導することで、Dll4/Notchシグナルを増強する。さらに、増強されたNotchシグナルは血管の基底膜の形成を誘導することで、血管を安定化することを示した。この研究成果は基礎医学のみならず臨床医学の点からも大変意義があり、今後の発展が期待できる。審査員は全員一致で学位に値するものと認める。