論　文　内　容　の　要　旨
Synopsis of Thesis

氏　名 Name	藤原　貴史
論文題名 Title	L-Arginine Stimulates Fibroblast Proliferation through the GPRC6A-ERK1/2 and PI3K/Akt Pathway (L-アルギニンはGPRC6A-ERK1/2およびPI3K/Akt経路を介して線維芽細胞の増殖を促進する)

論文内容の要旨

〔目　的(Purpose)〕

L-Arginine is considered a conditionally essential amino acid and has been shown to enhance wound healing. However, the molecular mechanisms through which arginine stimulates cutaneous wound repair remain unknown. Here, we evaluated the effects of arginine supplementation on fibroblast proliferation, which is a key process required for new tissue formation. We also sought to elucidate the signaling pathways involved in mediating the effects of arginine on fibroblasts by evaluation of GPRC6A, Akt, ERK1/2, and CREB activation.

〔方法ならびに成績(Methods/Results)〕

We first examined whether treatment with L-arginine affected fibroblast proliferation. Following 24 h of L-arginine starvation, NIH3T3 and primary human dermal fibroblasts (HDF) were treated with various concentrations of L-arginine (0-7 mM) for 24 h, and cell viability was then assessed. Six millimolar L-arginine produced maximum stimulation of proliferation.

We next determined whether L-arginine played an important role in preventing apoptosis by examining DNA fragmentation as a marker of apoptosis in the presence or absence of arginine. L-Arginine-deprived NIH3T3 and HDF demonstrated a significant increase in the percentage of terminal deoxynucleotidyltransferase-mediated dUTP-biotin nick end labeling (TUNEL)-positive cells compared with cells maintained in 6 mM arginine.

To examine the molecular mechanisms responsible for the proliferative effects of L-arginine in fibroblasts, we evaluated arginine-dependent activation of the ERK1/2, PI3K/Akt and cAMP-PKA pathway, which plays a major role in regulating cell growth, survival, and differentiation. HDF were deprived of L-arginine for 24 h and then supplemented with 6 mM L-arginine for different times (0-30 min). We found that L-arginine supplementation significantly increased levels of phosphorylated ERK1/2, Akt and PKA at 5-15 min. we next examined the effects of arginine on CREB phosphorylation. Similar to the effects observed for ERK1/2, CREB phosphorylation was also significantly increased at 5, 15, and 30 min with L-arginine.

Since GPRC6A is known to be a receptor for amino acids for basic amino acids such as L-lysine, L-arginine and L-ornithine, we speculated that GPRC6A would be stimulated by L-arginine to activate ERK1/2, Akt, PKA, and CREB. Therefore, we used siRNA to knockdown GPRC6A in HDF to investigate the activation of ERK1/2, Akt, PKA, and CREB. As a result, activation of ERK1/2, Akt, PKA, and CREB were all inhibited due to the knockdown of GPRC6A. In conclusion, we believe that GPRC6A resides in the upper stream of ERK1/2, Akt, PKA, and CREB to function as the receptor of L-arginine to regulate the cellular signaling.

〔総　括(Conclusion)〕

The present experiments demonstrated a critical role for the GPRC6A-ERK1/2 and PI3K/Akt signaling pathway in arginine-mediated fibroblast survival. Our findings provide novel mechanistic insights into the positive effects of arginine on wound healing.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

本研究は創傷治癒に重要である線維芽細胞の増殖に及ぼすアルギニンの効果について分析を行ったものである。NIH 3T3及びヒト皮膚線維芽細胞を各実験に用い、まずアルギニン刺激及び除去による線維芽細胞の増殖とアポトーシスを、MTS法とTUNEL染色によって評価している。その結果、アルギニン刺激により線維芽細胞の増殖促進、及びアポトーシス誘導の抑制を認めている。次に、様々な細胞の増殖に関わる重要なシグナル伝達分子であるERK1/2、PI3K/Akt、cAMP-PKAとそれらのターゲット分子であるCREBのアルギニン刺激による活性の変化をWestern blottingで解析するとともに、ERK1/2、Akt、PKAを阻害することによるアルギニン刺激における細胞増殖への影響をMTS法で解析した結果、アルギニンはERK1/2およびPI3K/Akt経路を介して線維芽細胞の増殖を促進させ、またこれらの上流にあるレセプターとしてGPRC6Aが存在することを明らかにしている。本研究は博士(医学)の学位を授与するに値するものと認める。