論 文 内 容 の 要 旨
Synopsis of Thesis

氏 名 Name	王 薇
論文題名 Title	Acromesomelic Dysplasia, Type Maroteaux Caused by Novel Loss-of-Function Mutations of the NPR2 Gene: Three Case Reports（新規NPR2遺伝子機能喪失変異による遠位中間肢異形成症マラト一型：3症例）

論文内容の要旨

〔目 的(Purpose)〕

The C-type natriuretic peptide (CNP)–natriuretic peptide receptor 2 (NPR2) signaling pathway plays an important role in chondrocyte development. Compound heterozygous or homozygous mutations of NPR2 have been found to be responsible for acromesomelic dysplasia, type Maroteaux (AMDM). However, the characterization of NPR2 mutants that cause AMDM has been limited in number. In the current study, we present three additional individuals with the AMDM phenotype caused by compound heterozygous or homozygous loss-of-function mutation in the NPR2 gene along with detection of functional changes of the NPR2 mutants.

〔方法ならびに成績(Methods/Results)〕

Method: The NPR2 gene was sequenced in three Korean patients with AMDM. An *in vitro* functional assay was performed to investigate the pathogenic significance of the three mutations (Tyr411His, Arg921X, and Arg921Gln). The HA-wt or HA-mutant constructs were constructed and transfected into HEK293A cells and whole-cell extracts were harvested for immunoblot analysis. The cGMP production was also examined with the treatment of CNP. To clarify the mechanisms for the absent or low response to CNP in the three loss-of-function mutations, we performed deglycosylation experiments and analyzed the subcellular localization of the HA-NPR2 mutants.

Results: Five novel NPR2 mutations were found in the three patients: two compound heterozygous mutations [c.1231T>C (Tyr411His) and c.2761C>T (Arg921X) in Patient 1 and c.1663A>T (Lys555X) and c.1711-1G>C (M571VfsX12) in Patient 3] and a homozygous mutation [c.2762G>A (Arg921Gln) in Patient 2]. Serum NT-proCNP concentration was significantly increased in each patient compared to control subjects. Cells transfected with the expression vector of each mutant except those found in Patient 3 showed a negligible or a markedly low cGMP response after treatment with CNP. HA-tagged wild type (wt) and HA-mutant NPR2 were expressed at comparable levels: there were two bands of ~130 and ~120 kDa in wt and Arg921Gln, a single ~120-kDa band in Tyr411His, and a single ~110kDa in the nonsense mutant. With respect to subcellular localization, Arg921Gln as well as wt-NPR2 reached the cell surface, whereas Tyr411His and Arg921X mutants did not. The Tyr411His and Arg921X NPR2 proteins were co-localized with an endoplasmic reticulum (ER) marker and failed to traffic from the ER to the Golgi apparatus. These results are consistent with deglycosylation experiments.

〔総 括(Conclusion)〕

In conclusion, we present three new patients of AMDM with five novel mutations of NPR2 genes. Our report indicates that Tyr411His and Arg921X NPR2 are complete loss-of-function mutations, whereas Arg921Gln behaves as a receptor with limited function.

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

骨系統疾患である acromesomelic dysplasia, type Maroteaux の３症例の解析を行い、*NPR2* 遺伝子に５変異を見いだした。

そのうちの３変異（Tyr411His, Arg921X, Arg921Gln）の機能解析を行い、Tyr411His, Arg921X は全く cGMP を産生する酵素活性を無くしているのに対して、Arg921Gln は低いながら残存酵素活性を有することを示した。さらに、細胞内局在の検討では Tyr411His, Arg921X は、小胞体に留まるのに対して、Arg921Gln は細胞膜に局在することを示した。さらに、NPR2 は N 型および O 型の糖鎖付加を受けるが、脱糖鎖酵素を使用した実験から、Tyr411His, Arg921X は、糖鎖付加を受けず、Arg921Gln は両方の付加を受けることを示した。これらは、見いだした変異が機能喪失型の変異であることを示しており、症例の原因であることを明確に示した研究であり、学位に値すると考える。