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博士の専攻分野の名称	博　士（医学）
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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	A genetic study of the effects of reduced Frs2a gene dosage in exostoses development and cartilage abnormalities observed in Ext2 heterozygous mutant mice （Ext2ヘテロ変異マウスで観察される外骨腫発生と軟骨異常に対するFrs2a遺伝子量低下の影響に関する遺伝学的研究）
論 文 審 査 委 員	（主査） 教　授　松尾　　勲 （副査） 教　授　大薗　恵一　　教　授　吉川　秀樹

論 文 内 容 の 要 旨

〔　目　　的　〕

Hereditary Multiple Exostoses (HME) is an autosomal dominant disorder characterized by the occurrence of multiple benign cartilage-capped tumors, or exostoses, at the ends of long bones. HME is caused by mutations in human *EXT1* and *EXT2* genes, which are responsible for heparan sulfate (HS) chain biosynthesis. Accordingly, inactivation of one copy of the mouse *Ext2* gene led to the HME phenotype and aberrant cartilage (termed as nodules) development in the ribs. Given that HS is essential for activation of FGF signaling, down-regulation of FGF signaling by *EXT* mutation might trigger HME development. However, direct evidence demonstrating the role of FGF signaling in exostoses development has not yet been shown. Therefore, the present work was undertaken to investigate whether FGF signaling via *Frs2α*, which is a crucial mediator of FGF signaling, regulates exostoses and ectopic cartilage development in *Ext2* heterozygous mutant mice.

〔　方法ならびに成績　〕

Methods: A transgene-inserted *Ext2* mutant mouse line ($Ext2^{Tg}$) was used. $Ext2^{Tg/+}$ mice were crossed with $Frs2\alpha^{+/-}$ mice to obtain $Ext2^{Tg/+}$; $Frs2\alpha^{+/-}$ mice. Numbers and locations of exostoses in different bones were investigated by soft x-ray analysis of one year old mice. Exostoses histology was examined by Hematoxylin and Eosin (H&E) staining of paraffin sections. Numbers and sizes of ectopic cartilaginous nodules were examined histologically by microscopic observation of H&E stained paraffin sections of postnatal days 14 (P14) ribs.

Results: Both $Ext2^{Tg/+}$ and $Ext2^{Tg/+}$; $Frs2\alpha^{+/-}$ mice developed histologically defined exostoses in their endochondral bones. Ribs were predominantly affected in both genotypes (33.3% and 47.0% of all exostoses in single and double heterozygotes, respectively), followed by the femur (19.0% and 29.4%, respectively). In $Ext2^{Tg/+}$ mutants, 75.0 % of mice (n=12/16) displayed exostosis phenotypes (at least one exostosis), including 37.5% (6/16) of mice showing multiple exostoses. In $Ext2^{Tg/+}$; $Frs2\alpha^{+/-}$ mutants, 66.7% (n=10/15) of mice exhibited exostoses, including 26.7% (n=4/15) of mice with multiple exostoses. The frequencies of exostoses appearance were not

significantly different between *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mutants. [1.31 ± 0.30 (mean ± SE) and 1.13 ± 0.30 (mean ± SE) for *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ respectively (t-test, p>0.05)]. Therefore, we did not find a significant difference between *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mice in the incidence rates of exostoses. Next, in order to investigate whether the appearance of ectopic cartilaginous nodules was affected by *Frs2α* gene dosage, nodules in *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mutant mice at P14 were analyzed. *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mice showed a tendency to have higher numbers of nodules (2.63 and 3.58 nodules per rib for *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mutants respectively) with larger sizes (4.49 and 6.65 cells per nodule in a single section for *Ext2*$^{Tg/+}$ and *Ext2*$^{Tg/+}$; *Frs2α*$^{+/-}$ mutants respectively). These results indicate that reduced *Frs2α* gene dosage might accelerate the growth of ectopic nodules.

〔 総　　括 〕

Taken together, our results indicate that reduction of *Frs2α* gene-dosage promotes growth of disorganized chondrocytes but does not strongly affect the *Ext2*-dependent HME phenotype.

論文審査の結果の要旨

ヒト遺伝性多発性外骨腫は、ヘパラン硫酸鎖合成に必須な*EXT1*及び*EXT2*遺伝子の変異によることが報告されている。また、ヘパラン硫酸鎖は、Fibroblast Growth Factor (FGF)をはじめ様々な分泌性シグナル因子の共受容体として働くことが示されている。しかし、現在までにFGFシグナルの低下が外骨腫発症の原因となっているか不明であった。

Nasirujjaman氏による本論文では、外骨腫を発症する*Ext2*ヘテロ変異マウスにおいてFGFの細胞内シグナル伝達に必須な*FRS2*αの遺伝子量を半分に低下させることによって、外骨腫及び異常な軟骨細胞塊の出現にどのような影響がみられるか検討した。軟X線撮影及び組織学的な解析から、*FRS2*αの遺伝子量を半分に低下させるだけで、*Ext2*ヘテロ変異マウスで見られる異常な軟骨細胞塊の数及び成長がともに促進されていることを見いだした。

以上の知見は、ヘパラン硫酸鎖欠損によるFGFシグナルの低下が外骨腫形成に必須なプロセスであることを支持する結果である点、博士（医学）の学位授与に値すると考えられる。