Stomatin-mediated inhibition of the Akt signaling axis suppresses tumor growth

氏　　名	NOR IDAYU BINTI A.RAHMAN
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審査委員	主査　教授　縣　保年
	副査　教授　九嶋　亮治
	副査　教授　向所　賢一

別紙様式３（課程博士・論文博士共用）

論　文　内　容　要　旨

※整理番号	928	（ふりがな） 氏　　名	ノル　イダユ　ア　ラフマン Nor Idayu A. Rahman
学位論文題目	Stomatin-mediated inhibition of the Akt signaling axis suppresses tumor growth		

Background and purpose

Tumor growth and progression are complex processes caused by mutual interactions between cancer cells and their surrounding stroma including many types of cellular and acellular components. Among them, the direct intercellular communications play roles in the regulation of tumor behaviors. However, the regulatory molecular mechanism largely remains unclear.

To examine the mechanism, the *in vitro* coculture system in which prostate cancer LNCaP cells can directly contact with primary human prostate stromal cells (PrS cells) has been developed, and genes that are upregulated in prostate cancer LNCaP cells have been screened. *Stomatin* was identified as one of the upregulated genes. Stomatin is tethered to the inner side of the plasma membrane. Although stomatin has been reported to be involved in the maintenance of the cell morphology, particularly in red blood cells, little is understood about its function in cancer cells. Therefore, the purpose of this study is to reveal the role and function of stomatin in cancer biology.

Methods

Prostate cancer LNCaP cells and PC3M cells, which did not express stomatin, and 22Rv1 cells, which expressed a certain degree of stomatin, were mainly used in this study. Stomatin expression in cancer cells was analyzed by quantitative PCR (qPCR) and western blot. Cell proliferation and TUNEL assays were performed to examine the stomatin function at the cellular levels. Doxycycline (Dox)-inducible expression system was used to stably and temporally express stomatin in prostate cancer LNCaP and PC3M cells. Stomatin expression was transiently and stably knocked down in 22Rv1 cells by transfection of stomatin siRNA and shRNA, respectively. The *in vivo* xenograft tumor model was generated by subcutaneous injection of prostate cancer cells in immunosuppressed SCID mice. The function of stomatin in the Akt-related pathway was analyzed in western blot and immunohistochemistry. Protein interactions of stomatin were detected in immunoprecipitation using total cell lysates and GST-pull down assay using recombinant proteins. With informed consent in the form of opt-out, the resected and stored prostate cancer samples from the patients were examined in qPCR and immunohistochemistry. Patients' clinical information was also used for the analysis of prostate cancer recurrence.

（備考）１．論文内容要旨は、研究の目的・方法・結果・考察・結論の順に記載し、２千字程度でタイプ等を用いて印字すること。
２．※印の欄には記入しないこと。

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（続紙）

Results

The mRNA and protein levels of stomatin were confirmed to be increased in LNCaP cells cocultured with PrS cells. The Dox-induced expression of stomatin in LNCaP cells and PC3M cells strongly suppressed Ki67-positive proliferative cells and induced apoptotic cells, resulting in the almost complete inhibition of tumor cell increase *in vitro*. In the *in vivo* xenograft tumor model, the tumor growth was significantly retarded by induction of stomatin expression. In contrast, when stomtin expression was knocked down in 22Rv1 cells, cell proliferation was enhanced and apoptosis was impaired, leading to the rapid cell increase *in vitro* and tumor growth promotion in *in vivo*.

The Akt-related pathway is crucial for cell proliferation and survival. Stomatin was found to inhibit phosphorylation (activation) of Akt. The phosphorylation is mediated by phosphoinositide-dependent protein kinase 1 (PDPK1), and PDPK1 protein stability is maintained by its binding to heat shock protein 90 (HSP90). In the immunoprecipitation and protein-protein binding experiments, stomatin directly bound to PDPK1 competitively with HSP90, and thus, PDPK1 expression was reduced. Conversely, knockdown of stomatin in 22Rv1 cells increased PDPK1 expression and Akt activation.

In tumor samples surgically isolated from patients with prostate cancer, stomatin expression was significantly decreased in the samples with high Gleason scores. It was observed that in the samples, stomatin was highly expressed in prostate cancer cells in juxtaposition to stroma cells. Finally, lower expression of stomatin was associated with higher recurrence of prostate cancer after the operation.

Discussion

This study revealed the stomatin-mediated novel signaling pathway to suppress tumor growth and progression. Stomatin expression was elevated by the interaction between prostate cancer cells and prostate stromal cells *in vitro* and *in vivo*, which mimics the tumor microenvironment that occurs upon cancer cell invasion into the surrounding connective tissue. The mechanism by which stomatin suppresses tumor progression was dependent on the inhibition of Akt activity through the instability of PDPK1 expression, resulting in both the significant attenuation of cancer cell proliferation activity and the enhancement of apoptosis.

However, it remains unclear how stomatin expression is upregulated in cancer cells by the direct cell-to-cell contact with surrounding stromal cells. Revealing the mechanism to regulate stomatin expression might lead to the development of novel anti-cancer therapy via the stomatin-mediated tumor suppressive actions.

Conclusion

This study revealed that stomatin inhibited cell proliferation and enhanced apoptosis in tumor cells through reduction of PDPK1 expression and subsequent attenuation of the Akt activation, leading to the suppression of tumor growth.

別紙様式９（課程博士・論文博士共用）

学位論文審査の結果の要旨

整理番号	９２８	氏　名	Nor Idayu A. Rahman
論文審査委員			

（学位論文審査の結果の要旨）※明朝体１１ポイント、６００字以内で作成のこと

本論文では、がん細胞が間質細胞との接着により受ける影響を明らかにするために、前立腺がん細胞株 LNCaP 細胞を前立腺間質細胞と共培養し、LNCaP 細胞で発現が上昇した Stomatin の機能について検討を行い、以下の点を明らかにした。

１）LNCaP 細胞で Stomatin を過剰発現させると増殖が抑制され、細胞死が誘導された。
２）Stomatin を過剰発現する LNCaP 細胞を免疫不全マウスに移植すると腫瘍の成長が抑制され、細胞死が誘導された。
３）Stomatin は、Akt をリン酸化する PDPK1 と結合し、PDPK1-HSP90 複合体を解離させ、PDPK1 を不安定化することで Akt のリン酸化を阻害し、増殖を抑制した。
４）内在性に Stomatin を発現するがん細胞で、Stomatin をノックダウンすると増殖が亢進し、マウスの移植系で腫瘍の成長が亢進した。
５）悪性度の高い前立腺がん検体では Stomatin の発現が低下した。
６）Stomatin の発現が低い前立腺がん患者では、再発率が上昇した。

本論文は、間質細胞との接着により前立腺がん細胞で発現が上昇する Stomatin の機能について新たな知見を与えたものであり、また最終試験として論文内容に関連した試問を実施したところ合格と判断されたので、博士（医学）の学位論文に値するものと認められた。

（総字数５６９字）

（令和３年　８月２５日）