Epithelial membrane protein 1 promotes tumor metastasis by enhancing cell migration via copine-III and Rac1.

著者	Ahmat Amin Mohammad Khusni Bin
year	2019-03-08
その他の言語のタイトル	Epithelial membrane protein 1ががん転移を促進するのは、copine-?による Rac1活性化で細胞遊走が亢進するためである
学位授与機関	滋賀医科大学
学位授与年度	平成30年度
学位授与番号	14202甲第831号
URL	http://hdl.handle.net/10422/00012536

doi: https://doi.org/10.1038/s41388-018-0286-0

氏　　名	Mohammad Khusni Bin Ahmat Amin
学位の種類	博士（医学）
学位記番号	博士甲博士第831号
学位授与の要件	学位規則第４条第１項
学位授与年月日	平成３１年　３月　８日
学位論文題目	Epithelial membrane protein 1 promotes tumor metastasis by enhancing cell migration via copine-III and Rac1. （Epithelial membrane protein 1 ががん転移を促進するのは、copine-Ⅲによる Rac1 活性化で細胞遊走が亢進するためである）
審査委員	主査　教授　縣　保年
	副査　教授　九嶋　亮治
	副査　教授　遠山　育夫

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	838	（ふりがな） 氏　　名	もはまっど　ふすに　びん　あーまと　あみん Mohammad Khusni Bin Ahmat Amin
学位論文題目	Epithelial membrane protein 1 promotes tumor metastasis by enhancing cell migration via copine-III and Rac1. （Epithelial membrane protein 1 ががん転移を促進するのは、copine-III による Rac1 活性化で細胞遊走が亢進するためである）		

目的 (Purpose)

Cancer metastasis is the worst-case phenomenon in the cancer progression and responsible for approximately 90% of cancer-related death. The interaction between cancer cells and other types of cells in the tumor microenvironment is the principal key that regulates cancer metastasis. However, the molecular mechanism by which the cell-to-cell contact-mediated tumor microenvironment is involved in the regulation remains to be elucidated. The purpose of this study is to unveil the regulatory mechanism by focusing on the intercellular physical contact between prostate cancer cells and stromal cells.

方法 (Method)

The co-culture system between human prostate cancer LNCaP cells and human prostate stromal (PrS) cells was developed to mimic the tumor microenvironment. The differentially expressed genes were analyzed by the Affymetrix DNA microarray and compared between LNCaP cells co-cultured with PrS cells and cultured alone. Stable clones of cancer cells overexpressing epithelial membrane protein 1 (EMP1) were established. EMP1-LNCaP cells or parental LNCaP cells (1×10^7 cells) were implanted into the prostate gland of male BALB/c nude mice (7 weeks old) via orthotopic injection and administrated for 6 weeks. The histopathological analysis was carried out to examine the tumor formation in the prostate and the metastasis in the lymph nodes and lung. The migratory activity was investigated using the Boyden chamber and invasion assay. To identify the binding partner of EMP1, the tandem mass spectrometry was performed. The immunoprecipitation and immunofluorescent microscope assays were conducted to examine the interaction between EMP1, copine-III, Src and Vav2. The pull-down assay was performed to evaluate the Rac1 activity. Human prostate cancer samples were used to analyze the expression level of EMP1.

結果 (Results)

As the result from bioinformatics analysis after the DNA microarray assay, the

（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。

expression level of EMP1 was significantly up-regulated in LNCaP cells co-cultured with PrS cells, compared with LNCaP cells cultured alone. When EMP1-LNCaP cells were orthotopically implanted into the prostate gland of nude mice, the lymph nodes and lung metastases occurred, which was not observed by the implantation of parental LNCaP cells. The size of the tumors grown in the prostate was similar between the two types of LNCaP cells. EMP1 has four times membrane passing structure and mainly localizes on the plasma membrane. To explore which molecules contribute to the EMP1-induced pro-metastatic property in cancer cells, the intracellular binding partner of EMP1 was examined by the tandem mass spectrometry, resulting in the identification of copine-III. The migration and invasion assays revealed that EMP1 promoted cell migratory activity in not only prostate cancer cells but also breast and colon cancer cells. The activation of Rac1, which plays an important role in cell migration, was induced by EMP1. The activation required the EMP1–copine-III binding. Next, it was investigated how Rac1 was activated downstream of the EMP1–copine-III complex. The complex phosphorylated and activated Src, and then, activated Src induced the activation of Vav2, a guanine nucleotide exchange factor for Rac1, leading to the enhanced cancer cell migration and metastasis. As clinical implications, increased expression and invasive front localization of EMP1 were observed in the samples from the patients with higher Gleason scores of prostate cancer.

考察 (Discussion)

This study has shown the pro-metastasis of a surface membrane protein EMP1 in both *in vitro* and *in vivo* models. The novel discovery at the molecular level in this study was that EMP1 directly interacted with copine-III in the cytoplasmic region, and that the interaction induced the activation of the Src-Vav2-Rac1 axis, which suggests the underlying mechanism in the EMP1-enhanced metastasis. The finding is supported by the evidence that the expression of copine-III was significantly higher in metastatic prostate cancer and ovarian endometrioid adenocarcinoma in the microarray data sets from the ONCOMINE database. In addition, genome-wide association analysis showed that an SNP in the *copine-III* gene is associated with susceptibility to prostate cancer. With respect to clinical implications, the development of pharmacological inhibitors or inhibitory antibodies against EMP1 may have a positive insight in anti-cancer therapy, because the high expression of EMP1 was observed in several types of pro-metastatic cancers. This might be a challenging future research project.

結論 (Conclusion)

This study demonstrates the potential mechanism of EMP1 in cancer metastasis. EMP1 and its binding partner copine-III facilitate the activation of Src, Vav2, and Rac1, resulting in enhanced cancer cell migration and metastasis.

別紙様式８（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	８３８	氏　　名	Mohammad Khusni Bin Ahmat Amin
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

癌細胞の周辺組織への遊走や遠隔臓器への転移には、ストローマ細胞との相互作用が重要であると考えられているが、それに関わる分子やメカニズムは明らかではない。

本研究では、前立腺癌細胞の遊走や転移に関わる因子として、EMP1 という膜タンパク質と、その細胞内ドメインに結合する Copine-Ⅲを同定し、EMP1 の発現によって遊走活性が上昇する分子メカニズムについて検討を行い、以下の点を明らかにした。

1）ストローマ細胞との共培養によって、前立腺癌細胞株 LNCaP 細胞で発現が上昇する遺伝子として EMP1 を同定した。
2）EMP1 を過剰発現した LNCaP 細胞は遊走活性が上昇し、ヌードマウスに移植すると転移能を示した。
3）EMP1 の細胞内ドメインに結合する因子として Copine-Ⅲを同定し、Copine-Ⅲをノックダウンすると EMP1 の過剰発現による遊走活性が低下することを示した。
4）EMP1 の過剰発現により、Src、Vav2、Rac1 経路が Copine-Ⅲに依存して活性化され、遊走活性が上昇した。
5）前立腺癌組織において、侵襲性の高い癌サンプルで EMP1 が発現上昇していた。

本論文は、癌細胞の遊走活性や転移能における EMP1 と Copine-Ⅲの機能について新しい知見を与えたものであり、最終試験として論文内容に関連した試問を受け合格したので、博士（医学）の学位論文に値するものと認められた。

（総字数　５９２字）

（平成 31 年 1 月 30 日）