論　文　内　容　の　要　旨
Synopsis of Thesis

氏　　名 Name	香林 正樹
論文題名 Title	Exploring the potential of engineered exosomes as delivery systems for tumor-suppressor microRNA replacement therapy in ovarian cancer （卵巣癌に対するマイクロRNA補充療法における、エクソソームのキャリアとしての有用性について）

論文内容の要旨

〔目　的(Purpose)〕

Ovarian cancer is one of the malignant tumors that seriously threatens women's health worldwide. Considering pivotal roles of microRNA (miRNA) in ovarian cancer biology, tumor suppressor (TS) miRNA is an attractive target; however, clinical trials have failed due to the difficulties of miRNA delivery, and therefore the development of a novel drug delivery system (DDS) is warranted. Exosomes, which are small extracellular vesicles (30-150 nm) derived from a multivesicular body, are stable in circulation and selectively picked up by cancer cells, indicating they can serve as a miRNA carrier. The aim of this study is to pursue the possibility of exosomes as a carrier for miRNA replacement therapy for ovarian cancer (OC).

〔方法ならびに成績(Methods/Results)〕

First, exosomes were purified from primary-cultured omental fibroblasts from ovarian cancer patients mainly by iodixanol (OptiPrepTM) density gradient ultracentrifugation. miR-199a-3p was selected as TS miRNA and synthesized miR-199a-3p tagged with Alexa-488 was loaded to exosomes by electroporation. Treatment with 199a-3p-loaded-exosomes (M199-exosomes) drastically increased miR-199a-3p expression level in OC cell lines (CaOV3; 8592-, SKOV3; 67188-, and OVCAR3; 2280-fold). M199-exosomes suppressed c-Met expression, a direct target of miR-199a-3p, and thereby inhibited cell proliferation and invasion of OC cell lines. In a xenograft study, M199-exosomes showed higher retention than bare miR-199a-3p in the circulation, suggesting its stability. After intraperitoneal treatment, only inoculated tumors displayed DIR fluorescence, indicating its specificity to cancer. Accordingly, M199-exosomes drastically inhibited peritoneal dissemination in mice. Immunohistochemical analyses revealed diminished c-Met expression in cancer followed by the inhibition of ERK phosphorylation and MMP2 expression.

〔総　括(Conclusion)〕

In this study, we revealed as follows; 1. exosomes derived from fibroblasts can be successfully collected from omentum of ovarian cancer patients. 2. TS miRNA, miR-199a-3p, was successfully incorporated into exosomes, and exosomes encapsulating miR-199a-3p drastically inhibited peritoneal dissemination of ovarian cancer model mice, suggesting its therapeutic potential. Given that most ovarian cancer patients undergo omentectomy and thereby exosomes from omental fibroblasts can be obtained from those, engineered exosomes can be utilized as a drug delivery carrier for a future molecular-targeted therapies for ovarian cancer, which may lead to personalized medicine in the near future.

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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

卵巣癌の新規治療法としてマイクロRNA補充療法が近年注目されている。しかし、マイクロRNAの生体内での不安定さゆえに、同治療方法は実現困難なのが現状である。本研究では、生体内で細胞間のシグナル伝達としての機能をもつエクソソームが、マイクロRNA補充療法におけるマイクロRNAのキャリアとして有用であるかを検証した。

当院で卵巣腫瘍手術を行う患者から大網を摘出し、そこから線維芽細胞を回収し、その細胞培養上清から超遠心法と密度勾配法を併用してエクソソームを抽出した。エクソソーム内に腫瘍抑制マイクロRNAを電気穿孔法で封入し、そのエクソソームに腫瘍抑制効果があることを*in vitro, in vivo*でともに確認した。そしてこの確認により、エクソソームがマイクロRNAのキャリアとして有用であることを証明した。

当実験で証明した結果により、今後エクソソームを癌治療に応用できる可能性が示唆された。

上記の論文は博士（医学）の学位授与に値する。