

News Release

Title

Malignant extracellular vesicles derived from ovarian cancer cells facilitate peritoneal dissemination, and the vesicles can be promising targets for improving patient outcomes.

Key Points

- This is the first report identifying extracellular vesicles (EVs)-related mechanisms of peritoneal dissemination.
- MMP1 mRNAs in EVs are key molecules for facilitating peritoneal dissemination by inducing destructive phenotypes in mesothelial cells, which are the main component of peritoneal membrane.
- The EVs containing MMP1 mRNAs could be a strong prognostic biomarker because MMP1 was significantly correlated with prognosis in stage I ovarian cancer patients.

Summary

Professor Fumitaka Kikkawa (Department of Obstetrics and Gynecology), Associate Professor Hiroaki Kajiyama (Department of Obstetrics and Gynecology), and graduate student Akira Yokoi (Department of Obstetrics and Gynecology) along with their collaborators, Dr. Takahiro Ochiya, at National Cancer Center Research Institute (Division of Molecular and Cellular Medicine), and Dr. Tomoyasu Kato at National Cancer Center Hospital (Department of Gynecology), described a newly identified mechanism of peritoneal dissemination facilitated by extracellular vesicles (EVs).

Peritoneal dissemination of cancer cells is responsible for the high morbidity and mortality of ovarian cancer, but unfortunately, very little is known about the molecular mechanisms underlying this type of metastasis. Almost all cell types, including tumor cells, secrete extracellular vesicles (EVs) including exosomes, and tumor-derived EVs play an important role in cancer initiation and progression. Here, we identified the EVs involved in the process of peritoneal dissemination. A major barrier for cancer cell dissemination into the peritoneal cavity is the peritoneal membrane; therefore, the destructive effect of the EVs derived from ovarian cancer cells on the mesothelial cells is favorable for cancer progression. Our findings suggest that EVs act as important mediators of ovarian cancer metastasis. Furthermore, the EVs containing MMP1 mRNAs, which we called malignant EVs, may be a strong prognostic biomarker because MMP1 mRNA was significantly correlated with prognosis in tumor tissue of stage I ovarian cancer patients. The detection of the malignant EVs can contribute to early diagnosis or prediction of metastasis or recurrence. Another importance is that these findings will suggest alternative therapeutic strategies for MMPs. If the

technology for inhibition of the EVs is developed in the near future, we will be able to manage the extracellular MMPs in malignant EVs. Additionally, the blockade of the EVs may prevent metastases. Thus, a newly identified malignant EVs may be promising targets for improving patient outcomes.

Research Background

Ovarian cancer is the most lethal gynecological malignancy, and the lethality mainly attribute to the frequency of peritoneal metastasis. Peritoneal dissemination of cancer cells is responsible for the high morbidity and mortality not only of ovarian cancer but also of gastrointestinal cancer. Unfortunately, the prognosis of cancer patients with peritoneal dissemination has not improved over the last several decades, and furthermore, very little is known about the molecular mechanisms underlying this type of metastasis.

Almost all cell types, including tumor cells, secrete EVs, and tumor-derived EVs play an important role in cancer initiation and progression by carrying various bioactive molecules. Over the last several years, the biological functions of EVs related to tumor metastasis have been investigated, and here, we identified for the first time the EVs involved in the process of peritoneal dissemination.

Research Results

It was clearly demonstrated that EVs derived from high metastatic ovarian cancer cells, ES-2 cells, promote peritoneal dissemination *in vivo*. Next, it was found that the mesothelial cells which are main component of peritoneal membrane, were structurally broken by treatment with the EVs from ES-2 cells *in vitro* and *in vivo* because of inducing apoptosis by the malignant EVs. From microarray gene expression analysis, MMP1 gene was significantly upregulated in mesothelial cells which treated with malignant EVs. Thus, MMP1 was selected and validated by qRT-PCR at high reproducibility. Interestingly, MMP1 mRNAs also packaged in the EVs from ES2, and it was confirmed that the gene was a key molecule for the destructive phenotypes. MMP1 was confirmed as a strong prognostic factor for early ovarian cancer patients according to open access database. In addition, the EVs which contained MMP1 mRNAs also existed in the EVs derived from ovarian cancer patients' ascites and furthermore the EVs also induced apoptosis *in vitro*.

Research Summary and Future Perspective

The current study identified EV-related mechanisms of peritoneal dissemination. The evidence for the involvement of EVs is clearly demonstrated by *in vitro* and *in vivo* experiments, and the existence of malignant EVs, which contain MMP1 mRNAs and facilitate peritoneal dissemination, was verified in clinical samples. The malignant EVs may be a strong prognostic biomarker because MMP1 mRNA is significantly correlated with prognosis in stage I ovarian cancer patients. The

detection of the malignant EVs can contribute to early diagnosis, prediction of metastasis and that of recurrence. The fact that MMP1 mRNA is packaged in EVs will contribute to drug discovery research because the research for MMPs is slightly stagnant due to the many previous disappointing clinical trial results by directly inhibiting MMPs. We believe that our findings will suggest alternative therapeutic strategies for MMPs. If the technology for inhibition of the EVs is developed in the near future, we will be able to manage the extracellular MMPs in EVs by indirect methods. Additionally, the blockade of the malignant EVs may prevent metastases. Thus, a newly identified malignant EVs may be promising targets for improving patient outcomes.

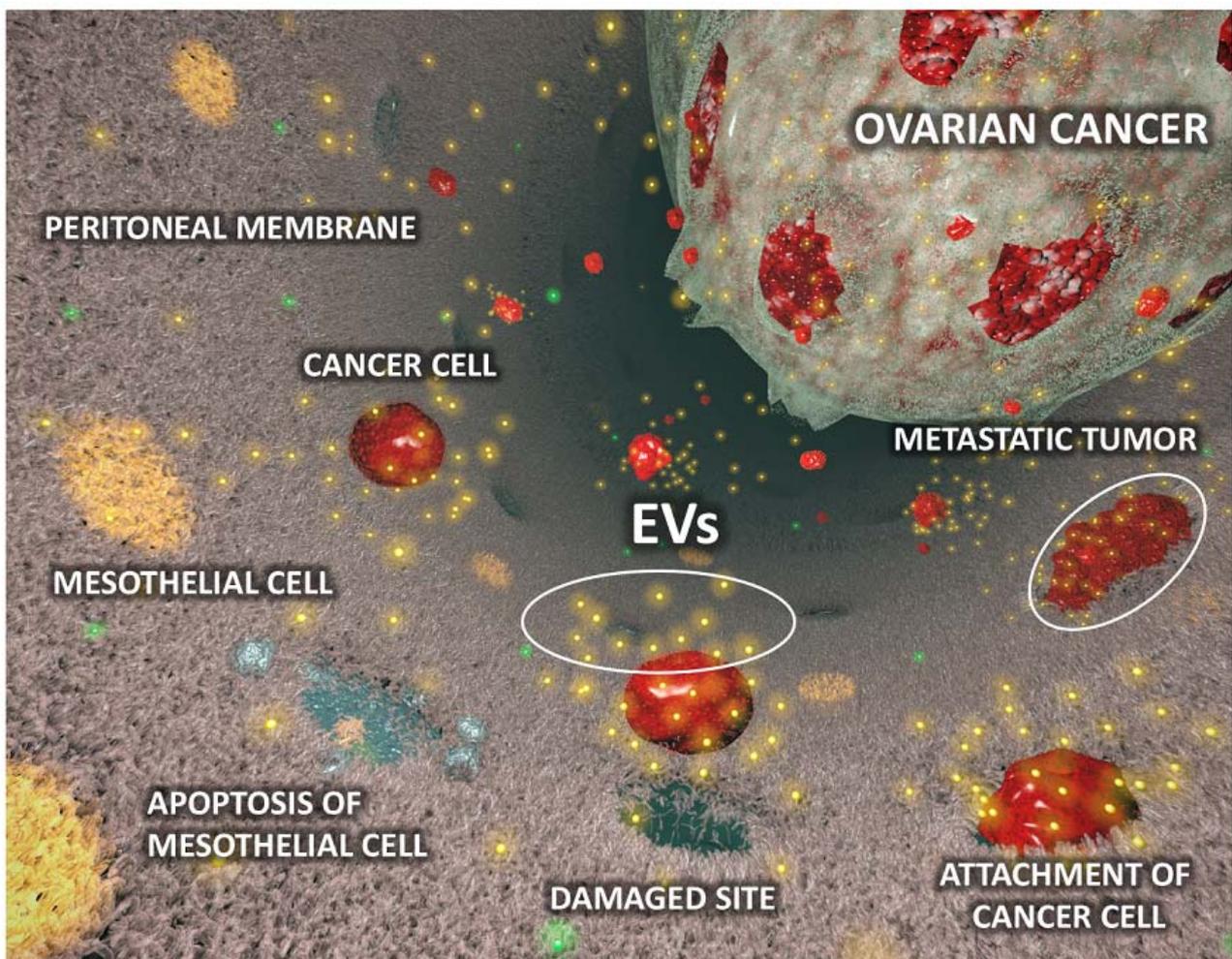


Figure: The diagram of peritoneal dissemination involved in extracellular vesicles (EVs) derived from ovarian cancer cells

Publication

Akira Yokoi, Yusuke Yoshioka, Yusuke Yamamoto, Mitsuya Ishikawa, Syunichi Ikeda, Tomoyasu Kato, Tohru Kiyono, Fumitaka Takeshita, Hiroaki Kajiyama, Fumitaka Kikkawa and Takahiro Ochiya. Malignant extracellular vesicles carrying MMP1 mRNA facilitate peritoneal dissemination in ovarian cancer. *Nature Communications*, Mar.6, 2017.

Japanese ver.

http://www.med.nagoya-u.ac.jp/medical/dbps_data/_material/_nu_medical/_res/topix/2016/mmp1_20170228jp.pdf