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Title	EMT-driven cancer malignancy: what is the fundamental matter? [an abstract of entire text]
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Description	この博士論文全文の閲覧方法については、以下のサイトをご参照ください。 配架番号：2475 他
Degree Grantor	北海道大学
Degree Name	博士(医学)
Dissertation Number	甲第13461号
Issue Date	2019-03-25
Doc URL	https://hdl.handle.net/2115/74737
Type	doctoral thesis
File Information	Haruka_Handa_summary.pdf



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博士の専攻分野の名称 博士（医 学） 氏 名 半田 悠

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Introduction:

In spite of world-wide research on cancer for almost half a century, cancer is still difficult to cure. Tumour metastasis is the most major cause in cancer-related death over the world. To understand the entity of tumour metastasis paves the way to more efficient treatment. The term 'metastasis' is generally used to describe the spread of cancer cells to other distant organs. First step of metastasis is invasion to extracellular matrix followed by intravasation, circulation, and extravasation. Enormous evidences have been shown that epithelial-mesenchymal transition (EMT) is highly responsible for cellular invasiveness, being not indispensable as some studies reported. Since It had been reported that mesenchymal cells secrete matrix metalloproteinases to negotiate the ECM barriers, the blockade of proteinase should have been an effective method to halt invasion, and thus metastasis. However, cancer cells have another route to execute metastasis, which is known for an amoeboid invasion. It has been generally accepted that most cancer cells addict aberrant signalling cascades which are attributed to genetic mutations of signal transduction molecule and inundatory amounts of ligands by tumour microenvironment. Professor Sabe's group linked growth factor signalling (e.g., receptor tyrosine kinases and lysophosphatidic acid receptor) and invasive machinery, in which Arf6, AMAP1 and EPB41L5 are cooperated with each other. Activated RTKs or GPCRs recruit Arf6, a small GTPase functioning in intercellular trafficking, and then the effector protein, AMAP1, binds to Arf6 in order to promote integrin recycling or EPB41L5-mediated E-cadherin internalization. This pathway is well-documented by Sabe's group in various types of cancer cells including breast, lung, head and neck, and renal cancer. Recent publication from this group states that malignant tumour cells arrange mitochondria geometry for preventing a catastrophe resulting from reactive oxygen species (ROS). These reports were focused mainly on mesenchymal invasion, and thus the relation between Arf6-pathway and amoeboid invasion has attracted my attention.

Some studies have indicated that EMT is involved in cellular stemness, that is, dedifferentiation programme. Therefore, mesenchymal cells *via* EMT have similar characteristics such as elongated phenotype, less cell-cell adhesion, anoikis resistance, and high motility, but not the same. Seminal papers demonstrated that p53 mutation can cause E-cadherin downregulation through suppressing certain microRNAs (miRNAs) against Zeb1. Our recent study revealed that p53-miRNA axis is not the sole mechanism for maintaining epithelial integrity. AMAP1 is an essential and implement protein of cellular invasion, so *bona fide* mesenchymal cells highly express AMAP1, while epithelial cells do not express. The question has been addressed whether p53 regulate AMAP1 expression levels depending on cell lineage or not.

EMT induces diverse alterations from gene transcription to cytoskeletal remodelling, and probably metabolic reprogramming. Previous study in our laboratory found that normal epithelial cells can enhance oxidative phosphorylation (OXPHOS) capacity accompanied with mitochondrial fission. Generally considered that mitochondrial fission impairs OXPHOS activity through cristae destruction, these data were surprising. In this thesis, I attempt to elucidate the molecular mechanism of this phenomenon and to answer its biological question.

Studies on this thesis were conducted in three different approaches;

- (1) To clarify the role of Arf6-AMAP1-EPB41L5 pathway in cancer invasion
- (2) To investigate the p53 role in regulating AMAP1 expression in epithelial cells
- (3) To unveil the relation between EMT and OXPHOS in normal and transformed cells

Material and Methods:

(1) Amoeboid invasion was induced by using protease inhibitors in MDA-MB-231 breast cancer cells which have high expression levels of Arf6-AMAP1 pathway components. Then, they were cultured on the top of collagen to assess their ability of amoeboid invasion. Silencing of each component of Arf6-pathway was conducted to evaluate their role in amoeboid invasion.

(2) Microarray analyses on miRNA expression among mutant-p53 (mt-p53), silenced-p53 (shp53), and wildtype-p53 (wt-p53) were performed to seek the causative miRNA. miRNAs which had the negative correlation between AMAP1 were searched by using The Cancer Genome Atlas (TCGA) database. Reporter assay demonstrated that certain miRNAs can bind to 3' UTR of AMAP1 mRNA. The expression levels of these miRNAs and AMAP1 were also assessed in non-transformed mammary epithelial cell line, breast cancer cell line harbouring wt-p53, and *bon fide* mesenchymal cell line. The Encyclopedia of DNA elements (ENCODE) database was used for analysing chromatin status among normal epithelial cells, fibroblasts and another type of cell.

(3) Oxygen consumption rate (OCR) was measured between normal mammary gland cells with or without TGF-beta. The protein of interest was found through analysing gene expression omnibus (GEO) datasets. The causative protein was overexpressed in cells and then OCR was assessed. The expression levels of this protein among normal and cancer cell lines were also examined by immune-blotting.

Results:

(1) Arf6-AMAP1-EPB41L5 pathway activated by either RTK or GPCR is closely related to amoeboid-invasion.

(2) AMAP1 expression is downregulated via miR-182/-96 which are highly expressed in epithelial cells harbouring wt-p53. p53 has an access limitation in certain miRNAs' cluster, which may lead to a proper function of epithelial cells and professional mesenchymal cells.

(3) A protein that is underregulated after EMT was sufficient for the enhancement of OXPHOS capacity during EMT in non-transformed epithelial cells.

Discussions and Conclusions:

The body of work is conducted to clarify the entity of EMT. Our previous studies have shown that the invasiveness driven by Arf6-AMAP1 pathway could be targeted by statins. Although clinical trials targeting matrix metalloproteinases were not efficient because of provoking amoeboid invasion, Arf6-pathway is the common pathway of mesenchymal- and amoeboid- invasion and therefore statin treatment will potentiate cancer therapy.

The results from the second theme insists that p53 is just a guardian against abnormal cellular functions. The molecular basis of how the epigenetic status of certain locus are destined is pretty challenging and should be under intense scrutiny.

OCR is a surrogate for OXPHOS ability of generating ATP, and thus the actual production of cellular ATP has to be examined.

These findings in this thesis push back our boundaries to understanding what is EMT, and can contribute to our human society in near future.