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# 学位論文内容の要旨

博士の専攻分野の名称　博士（医　学）　氏 名　半田　悠

学 位 論 文 題 名

EMT-driven cancer malignancy: what is the fundamental matter?

（EMT とがん悪性度：何が根本的事象なのか。）

**Background and Objectives:**

Tumour metastasis is the main cause of cancer-related death worldwide. Understanding the entity 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. The first step of metastasis is invasion to the extracellular matrix (ECM), followed by intravasation, circulation and extravasation. Much evidence has shown that epithelial–mesenchymal transition (EMT) is highly responsible for cellular invasiveness, but is not indispensable, as reported by some studies. As it has been reported that mesenchymal cells secrete matrix metalloproteinases to negotiate the ECM barriers, blockading proteinase should be an effective method of halting invasion and thus metastasis. However, cancer cells metastasise through another route, known as amoeboid invasion. It is generally accepted that most cancer cells have aberrant signalling cascades, which are attributed to genetic mutations of signal transduction molecules and ligand inundation by the tumour microenvironment. Professor Sabe’s group linked growth factor signalling (e.g. receptor tyrosine kinases [RTKs] and lysophosphatidic acid receptor) and invasive machinery, in which Arf6, AMAP1 and EPB41L5 cooperate with each other. Activated RTKs or GPCRs (G protein–coupled receptors) recruit Arf6, a small GTPase (guanosine triphosphatase) functioning in intercellular trafficking, and then the effector protein AMAP1 binds to Arf6 to promote integrin recycling or EPB41L5-mediated E-cadherin internalization. This pathway has been well-documented by Professor Sabe’s group in various cancer cell types including that of breast, lung, head and neck and renal cancer. These reports mainly focus on mesenchymal invasion, and thus the relationship between the Arf6 pathway and amoeboid invasion has sparked my interest.

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

EMT induces diverse alterations from gene transcription to cytoskeletal remodelling, and probably induces metabolic reprogramming. In a previous study, our laboratory found that normal epithelial cells can enhance their oxidative phosphorylation (OXPHOS) capacity accompanied by mitochondrial fission. Generally, considering 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 the biological question.

The studies in this thesis were conducted using three different approaches:
(1) To clarify the role of the Arf6–AMAP1–EPB41L5 pathway in cancer invasion
(2) To investigate the role of p53 in regulating *AMAP1* expression in epithelial cells
(3) To unveil the relationship between EMT and OXPHOS in normal and transformed cells

**Material and Methods:**
(1) Protease inhibitors were used to induce amoeboid invasion in MDA-MB-231 breast cancer cells, which have high expression levels of the Arf6–AMAP1 pathway components. Then, the cells were cultured on collagen to assess their amoeboid invasive ability. Each component of the Arf6 pathway was silenced to evaluate its role in amoeboid invasion.
(2) miRNA expression in mutant p53 (mt-p53), silenced p53 (sh-p53) and wild-type p53 (wt-p53) was analysed using microarray to seek the causative miRNA. Reporter assay demonstrated that certain miRNAs can bind to the 3′ untranslated region (3′ UTR) of *AMAP1* mRNA. The expression levels of these miRNAs and *AMAP1* were also assessed in a non-transformed mammary epithelial cell line, a breast cancer cell line harbouring wt-p53, and a *bona fide* mesenchymal cell line.
(3) The oxygen consumption rate (OCR) between normal mammary gland cells (NMuMG cells) with or without transforming growth factor-beta (TGF-β) was measured. The protein of interest was found by analysing Gene Expression Omnibus (GEO) datasets. The causative protein was overexpressed in NMuMG cells, and then the OCR was assessed. In addition, the expression levels of the protein of interest in normal and cancer cell lines were examined by immunoblotting.

**Results:**
(1) The Arf6–AMAP1–EPB41L5 pathway, activated by either RTKs or GPCRs, is closely related to amoeboid invasion.
(2) p53 is restricted to access in certain miRNA clusters, which may lead to proper function of epithelial cells and professional mesenchymal cells.
(3) A protein that is under-regulated after EMT was sufficient for enhancing OXPHOS capacity during EMT in non-transformed epithelial cells.

**Discussion and Conclusions:**
This work was conducted to clarify the entity of EMT. Our previous studies have shown that statins can target Arf6–AMAP1 pathway–driven invasiveness. Although clinical trials targeting matrix metalloproteinases were not efficient because they provoked amoeboid invasion, the Arf6 pathway is the common pathway of mesenchymal and amoeboid invasion and therefore statin treatment would potentiate cancer therapy.
The results from the second approach confirm that p53 only guards against abnormal cellular functions. The molecular basis of how the epigenetic status of certain loci are destined is quite challenging and should be intensely scrutinised.
OCR is a surrogate for the ability of OXPHOS to generate adenosine triphosphate (ATP), and thus the actual production of cellular ATP should be examined.
The findings in this thesis expand the boundaries of our understanding of what EMT is, and can contribute to human society in the near future.