

[Title]

Visualization of a cancer metastasis mechanism at nanometer level: Discovery of dramatic changes of membrane dynamics in cancer cells during metastasis

[Summary]

A research group led by Professor Noriaki Ohuchi, Senior Assistant Professor Kohsuke Gonda at Graduate School of Medicine, Tohoku University and Professor Hideo Higuchi at Graduate School of Science, The University of Tokyo has developed an optical system to image with a spatial precision of 9 nanometer *in vivo*. The optical system enables to visualize protein and drug at single molecular level in tumor-bearing mice which is implanted with human breast cancer cells. The most important biological property of cancer is its ability to spread to other organs. The research group labeled the metastasis-promoting protein on the cell membrane with fluorescence particle and has analyzed the protein dynamics with the newly developed optical device. In this study, they firstly discovered following cancer mechanisms using mice:

1. A change of cell morphology is important for cancer metastasis.
2. Cancer cells showed increases in migration speed (diffusion speed) of membrane protein (over 1000-fold) with progression of metastasis. The change of migration speed is important for cancer cell to metastasize.

A cancer metastasis mechanism at molecular level has long been unknown because a spatial precision of previous *in vivo* imaging was at micrometer level. This study enables to visualize the mechanism of cancer metastasis at molecular level. The results are expected to clarify an activation mechanism of cancer metastasis, evaluate malignant grade by measuring membrane protein migration speed, and develop a new treatment with improved anticancer drug. This report was published online in *Journal of Biological Chemistry* (vol. 285, 2750-2757), a Life Sciences journal, on January 22, 2010. The paper's title is "*In vivo* nano-imaging of membrane dynamics in metastatic tumor cells using quantum dots".

[Details]

The leading cause of death is cancer in Japan, and more than 330,000 nationals died of it for the year. The most important biological property of cancer is its ability to spread to other organs. The society has a high expectation of the clarification of cancer metastasis mechanism and the development of a diagnosis system and a treatment. It is important for understanding of cancer metastasis that the dynamics of cancer-activating protein could be more precisely analyzed in tumor-bearing mice. However, the spatial precision of previous *in vivo* imaging is limited to the micrometer level, and it is not enough to analyze protein or drug due to their size (1-100 nm). Thus, further development of *in vivo* imaging with high spatial precision has been expected. The research group has developed the optical system that can analyze protein dynamics in mice with 9 nanometer-precision (Fig. 1). To perform high-precise imaging, a fixation method of mice reducing biological oscillation such as aspiration or pulsation was improved, and a position analysis method of fluorescently-labeled protein was developed (Fig. 1).

The previous studies using cultured cells (*in vitro*) showed that cancer cell migration capability and an increase in membrane protein migration speed closely related to the activation of cancer metastasis. In migration of cancer cells *in vitro*, the cells form the pseudopodia, which is similar to membrane protrusion structure, in the direction of migration. The structure is the driving force of cell migration. Greater membrane protein

migration speed is thought to accelerate a reaction rate between activation signals of cancer metastasis and its receptor (membrane protein). However, these results were provided by only *in vitro* experiments separated from the body. Therefore, it has been expected to study cancer cells in human or mice tumor which contains blood vessels and complicated tissue structures. The research group has focused on a metastasis-promoting factor on the cell membrane, protease-activated receptor 1 (PAR1), and prepared anti-PAR1 antibody to identify specifically PAR1 on cancer cell membrane (Fig. 2A). The PAR1 in cancer cells in mice was fluorescently-labeled by tail vein injection of tracer, a PAR1 antibody-conjugated quantum dot (fluorescent nanoparticles) (Fig. 2A and B). The research group analyzed a barycentric position of fluorescent quantum dots to measure PAR1 movements by optical system developed (Fig. 1). In the imaging of mice, the membrane dynamics of metastatic cancer cells in four regions was observed: far from the blood vessel in cancer tissues, near the vessel, in the bloodstream, and adherent to the inner vascular surface in normal tissues near tumors (Fig. 2C-G). These locations represent the process of cancer metastasis. The results showed that PAR1 in cancer cells far from blood vessels moved extremely slowly (Fig. 2C), and that the PAR1 migration speed increased during intravasation (Fig. 2C-E). The speed in cancer cells in the bloodstream became more than 1000-fold greater than that far from vessels (Fig. 2C and E). It is thought that an increase in membrane protein migration speed activates cancer metastasis. When cancer cells in bloodstream adhered to vessel wall, PAR1 migration speed was 20-fold less than that in the bloodstream (Fig. 2C-G). The specific formation of pseudopodia in the direction of migration was observed in cells near the vessels (Fig. 2D) and adhering to the vessel wall (Fig. 2G). It is also clarified that PAR1 migration speed on pseudopodia was two-fold greater than that of other regions in the migrating cells (Fig. 2G).

These results show that cancer cells effectively activate proliferation and metastasis ability by changing a membrane protein migration speed depending on location in cells or tissues. The achievements are expected to clarify an activation mechanism of cancer metastasis, evaluate malignant grades by measuring membrane protein migration speed, and develop a new treatment with improved anticancer drug.

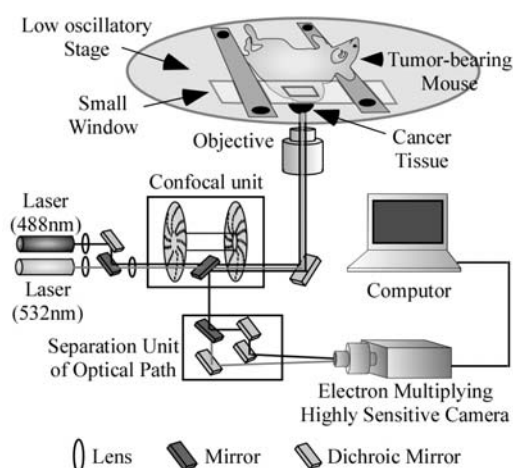


Figure 1. Optical system for *in vivo* imaging.

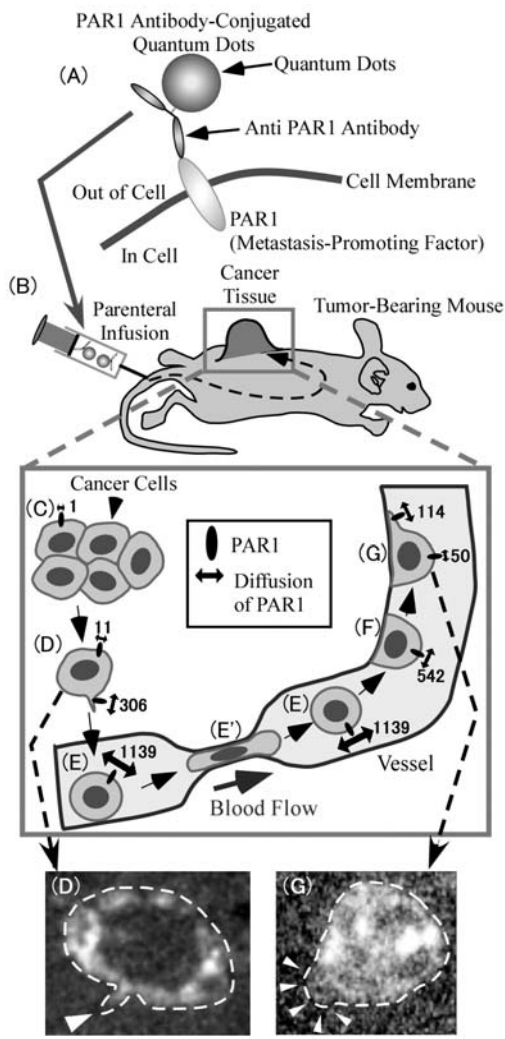


Figure 2. An outline for methods and results of *in vivo* imaging of cell membrane dynamics during cancer metastasis.

*C-G*, Cells *in vivo* during metastasis. *C*, Cells far from vessels. *D*, Cell near vessels. *E*, Cell in the bloodstream. *E'*, Cells in narrow vessels. *F*, Cells adhering to the inner vascular surface without directional migration. *G*, Cells migrating directionally on the surface. The number in *C-G* means the relative value when the migration speed of PAR1 in the cells far from vessels was defined as 1. The arrowheads in *D* and *G* indicate clearly-imaged pseudopodia. Bar, 10 micrometer (*D* and *G*). In conclusion, PAR1 migration speed of metastasizing cancer cells increases during intravasation, reaches a peak in the vessel, decreases at extravasation, and is also higher at locally formed pseudopodia. The dramatic changes in membrane protein and morphology enable cancer cells to metastasize.

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