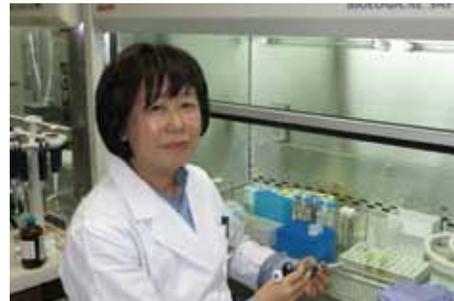


Challenge for CTCs analysis and tumor diagnosis with confocal image cytometer

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Circulating tumor cells (CTCs) are cells that break away from the primary tumor and circulate into the bloodstream. CTCs are known as metastatic seeds that are generated in the early stage of tumor formation. The department of Surgery at Teikyo University School of Medicine detected CTCs of digestive organ cancer using multiple markers, including EMT and tumor stem-like cells, and also showed the utility of CTCs as biomarkers for recurrence, prognosis and prediction of the treatment outcome. Dr. Hisae Inuma talked about her research and upcoming CTCs study.

Please tell us about your current research.

I am interested in tumor biomarkers capable of being effectively used for early diagnosis and prognosis (recurrence and metastasis). Especially, I am focusing on developing a low invasive diagnosis method(Liquid biopsy) for patients. A tumor is generally diagnosed by taking a part of its tissue. However, it causes physical burden on patients and takes time to confirm recurrence and metastasis. Meanwhile, CTCs(circulating tumor cells) are accessible and useful for early tumor diagnosis.

The utility of small CTCs as biomarkers for recurrence, prognosis and prediction of the treatment outcome has been shown by our studies. Moreover,

we found CTCs were biomarkers for epidermal cell, EMT(Epithelial-Mesenchymal transition)and tumor stem-like cells. Recently, we have been investigating microRNA in blood plasma exosome and evaluating its benefit as a biomarker for early diagnosis.

Why did you choose to use the CQ1?

CTCs are very rare cells in cancer patient's blood and are can be detected as noise with other instruments such as the flow cytometer. Therefore, we were trying to find an instrument which could accurately quantify small amounts of CTCs. At that time, we were informed of the confocal image cytometer CQ1 which can quantify multi-color samples, and we wanted to look into this further by CQ1.

What are CTCs (Circulating tumor cells)?:

CTCs are tumor cells which circulate in peripheral blood. Developed tumors metastasize through the bloodstream and lymph fluid. Therefore, tumor cells exist in the bloodstream when metastasis occurs. The detection of CTCs makes it possible to diagnose recurrence and metastasis at an early tumor stage. CTCs' numbers are very small as only less than 100 CTCs are contained in more than 1x10⁶ of blood cells in 10ml of cancer patient's blood. Therefore it is difficult to detect CTCs with a flow cytometer because they detect CTCs as noise. However, it is very easy to detect rare CTCs with an Image cytometer.

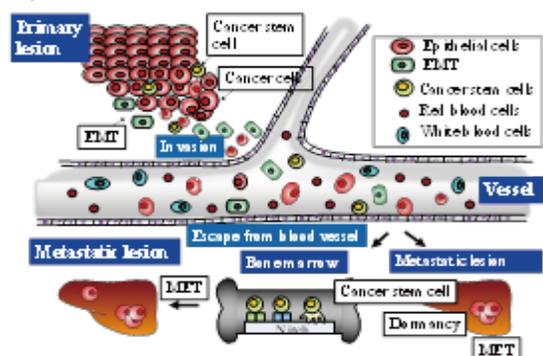


Fig 1 Tumor metastasis cascade (with hypothesis)

What do you think performance of CQ1?

In CQ1, we could detect accurate CTCs data effectively. We can automatically measure many samples within a short period of time. We were able to obtain very valuable data with CQ1 which is otherwise impossible to get with other instruments.

How will you use CQ1?

CTCs in patients express not only epithelial cells markers but also the molecules related to tumor stem-like cells, the EMT and the grade of the tumor. These molecules differ heterogenetically from primary site.

We intend to investigate the correlation between the treatment and biomarkers changes in tumor cells in an attempt to create an index for accurate individualized treatment.

Therefore, single CTC cell need to be isolated and analyzed for DNA mutation and RNA expression by next generation sequencing technologies.

Also, we are interested in culturing CTCs and analyzing 3D spheroids.

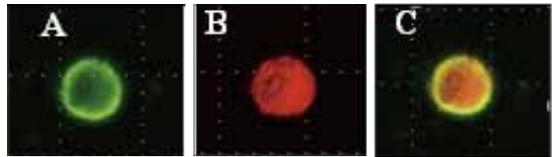
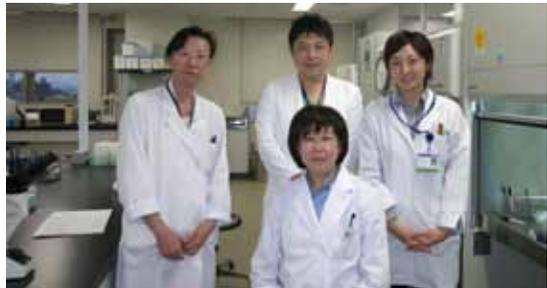


Fig 2 CTCs expression molecules

- A. Marker for epithelial cells (AE1/AE3: FITC label)
- B. Marker for tumor stem-like cells (CD133: Texas Red label)
- C. Marker for both epithelial cells and tumor stem-like cells (AE1/AE3*CD133)

Reference

- 1) Iinuma H, Watanabe T, Mimori K. et al. Clinical significance of circulating tumor cells, including cancer stem-like cells, in peripheral blood for recurrence and prognosis in patients with Dukes' stage B and C colorectal cancer. *J Clin Oncol* 29:1547-1555, 2011
- 2) Yokobori T, Iinuma H, Shimamura T et al. Platin3 is a Novel Marker for Circulating Tumor Cells Undergoing the Epithelial-Mesenchymal Transition and Is Associated with Colorectal Cancer Prognosis. *Cancer Res.* 73 (7): 2059-2069, 2013



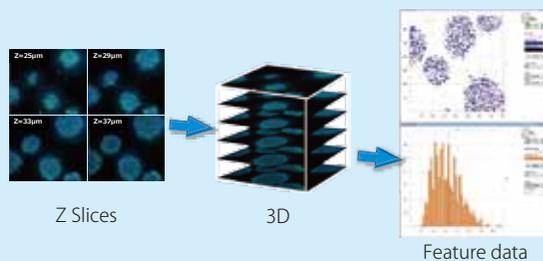
Cell clusters are directly measurement with high-throughput 3D imaging -Confocal Quantitative Image Cytometer CQ1-



- Enables measurement of spheroids, colonies and tissue sections
- Offers the similar operability to traditional FCM
- Open platform
- Compact footprint, light weight bench-top device; no need for darkroom

Application: 3D measurement of spheroids

Recognize each individual cell within a spheroid in 3D, to measure cell number and size. Heat-map display is possible depending on the data.



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