Abstract of the 2011th Maruyama Memorial Lectures of the 80th Annual Meeting of the Medical Association of Nippon Medical School

Date: September 1, 2012　Place: Nippon Medical School

The Abstract of the 2011th Maruyama Memorial Research Fund Prize Memorial Lecture

In situ Search for Breast Cancer Stem Cells and Their Niche: The Film Sheet Epoxy Resin Embedding Method and Breast Cancer Stem Cells

Yoichiro Yamamoto[1], Tatsuo Oguro[2], Junko Mieda[2],
Ryuji Ohashi[1] and Shinichi Tsuchiya[1]

[1]Division of Diagnostic Pathology, Nippon Medical School Hospital
[2]Division of Pathology, Nippon Medical School Chiba Hokusoh Hospital

Because of the significant increase in the number of cases, breast cancer has attracted much attention in Japan. Breast cancer now kills more than 10,000 Japanese women each year. Recent reports of small subgroup of cells called "cancer stem cells" that have self-renewal and differentiating abilities and are capable of forming tumors have caused a paradigm shift in our understanding of cancer[1]. For example, because cancer stem cells contain a type of ATP-binding cassette transporter, they are highly resistant to anticancer agents[2]. Furthermore, cancer stem cells are greatly affected by their "niche", and/or microenvironment[3]. We believe that by focusing on cancer stem cells and their niches, new treatments to suppress the recurrence and metastasis of tumor cells can be developed.

Both CD44+/CD24−/low and aldehyde dehydrogenase 1 are candidate markers for identifying cancer stem cells in breast cancer[4,5]. However, the location of breast cancer stem cells has not been identified. Therefore, we are using a new technology for *in situ* identification of breast cancer stem cells, the film sheet epoxy resin embedding method (FSEM)[6]. The FSEM is a new technology that allows nanoscale *in situ* observation with electron microscopy of target cells and the neighboring microstructure through comparison with hematoxylin and eosin (HE)-stained and immunohistochemical specimens (or *in situ* hybridization specimens **(Fig. 1)**. Basal clear cells identified with electron microscopy in the normal milk duct are candidate stem cells[7]. They lie at the base of the normal milk duct, contacting the basement membrane, and have a clear cytoplasm, a circular nucleus, and few cytoplasmic organelles. Because identification with standard light microscopy is difficult, we search cancer cells with the feature of basal clear cells extensively with the FSEM **(Fig. 2)**. Breast cancer cells appear relatively uniform on light microscopy. However, differences in cell size are clearly visible on electron microscopy. Furthermore, occasional cells have few cytoplasmic organelles.

A major advantage of the FSEM is that an electron micrograph can be acquired with positional information corresponding to an HE-stained specimen. Therefore, the FSEM has an affinity with digital pathology. We are

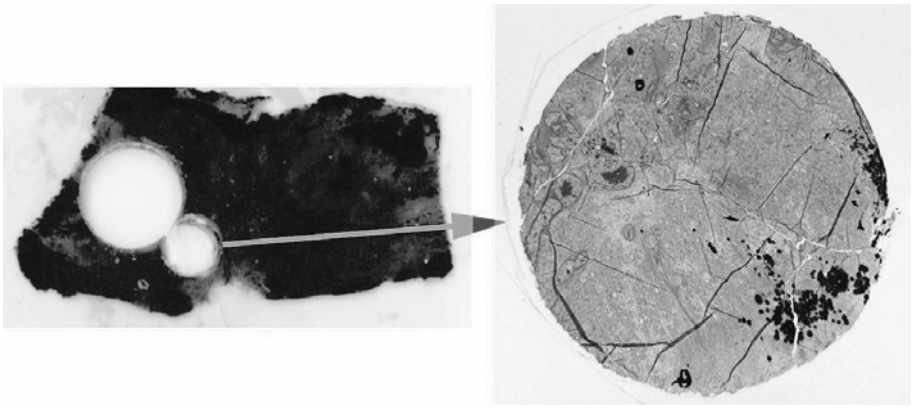

Fig. 1 Left: Clipping out of the region of interest from a piece of tissue sandwiched between film sheet layers. Right: Observing the clipped-out region.

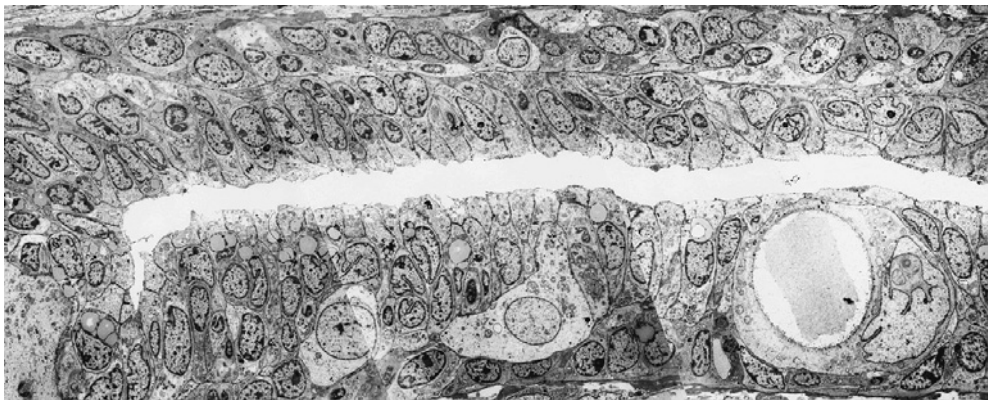

Fig. 2 Electron micrograph obtained with the FSEM. We can observe the region corresponding to the HE-stained specimen at the electron microscopic level.

pursuing this technology with the integration of virtual slides. In addition, we are also pursuing the development of an indicator—a stable marker for breast cancer stem cells—that can be used instead of CD44+/CD24−/low, whose staining ability is variable.

Today, molecular biological approaches, including genetic analysis, have become mainstream, and morphologic analysis is sometimes considered a relic of the past. However, morphologic analysis remains superior for searching for the structures of cancer stem cell niches and/or microenvironment. We believe that morphologic analysis can develop new approaches for cancer research.

References

1. Reya T, Morrison SJ, Clarke MF, Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 2001; 414: 105–111.
2. Dean M: ABC transporters, drug resistance, and cancer stem cells. J Mammary Gland Biol Neoplasia 2009; 14: 3–9.
3. Iwasaki H, Suda T: Cancer stem cells and their niche. Cancer Sci 2009; 100: 1166–1172.
4. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF: Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003; 100: 3983–3988.
5. Ginestier C, Hur MH, Charafe-Jauffret E, et al.: ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. Cell Stem Cell 2007; 1: 555–567.
6. Oguro T, Asano G, Ebina T, Watanabe K: Ultrastructural Characterization of the Role of Fibroblasts in Tumor Microvessel Networking. Japanese Journal of Clinical Electron Microscopy 2001; 33: 131–135 (in Japanese).
7. Joshi K, Smith JA, Perusinghe N, Monoghan P: Cell proliferation in the human mammary epithelium. Differential contribution by epithelial and myoepithelial cells. Am J Pathol 1986; 124: 199–206.