Morning Seminar I
Aromatase and Aromatase Inhibitors

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Aromatase inhibition provides both paracrine/intracrine and endocrine treatment. Recent accumulated data clarified that 3rd generation aromatase inhibitors potently suppress intratumoral estrogen synthesis particularly in postmenopausal patients. In the 2nd-line treatment for metastatic breast cancer patients, aromatase inhibitors achieved results antitumor activity at least equal to and sometimes better than that of tamoxifen. In the first-line treatment for metastatic breast cancer patients, a recent pivotal study clearly demonstrated that aromatase inhibitor was superior to tamoxifen. Based upon these results, various adjuvant trials which compare aromatase inhibitors with tamoxifen and attempt to determine optimal combination therapies and treatment periods with aromatase inhibitors are currently being conducted. In addition, preliminary studies conducted in neoadjuvant setting indicated that aromatase inhibitors showed an extremely high response rate, which predicts a future paradigm, that neoadjuvant therapy using aromatase inhibitors singly or in combination may become standard for hormone-responsive and post-menopausal breast cancer patients.

Key words: Aromatase, Aromatase inhibitor, Hormone therapy, Breast cancer, Microenvironment

Biology of Intratumoral Aromatase
Levels of estrogens, especially estradiol (E2), are elevated in breast tumor tissues compared with adjacent normal tissues with a gradient from the tumor to surrounding tissue in postmenopausal patients (Fig 1). Among the various mechanisms of peripheral estrogen synthesis, aromatase and 17β-hydroxysteroid dehydrogenase (HSD) are known to play major roles, because these enzymes are overexpressed selectively in tumor tissues. Successful therapeutic outcomes achieved using aromatase inhibitors confirmed that breast tumors are dependent considerably upon intratumoral estrogens for growth. Furthermore, it has been indicated that aromatase inhibitors suppress the development of tumors in rat carcinogenesis models. Immunohistochemical studies showed that aromatase is expressed mainly in fibroblastic cells and adipocytes in primary breast tumor tissues. Cultured media from breast cancer cells stimulated aromatase activity in fibroblastic cells, which suggests that aromatase activation is induced in a paracrine fashion. On the other hand, HSD expression was detected in both tumor cells and stromal cells in primary breast tumor tissues, indicating that the breast tumor microenvironment is regulated in a different manner.

Recently, two important studies have been published regarding the biological implications of aromatase in breast cancer. Kirmi et al. documented that overexpression of aromatase can lead to hyperplasia and alter the expression of genes involved in apoptosis, cell cycle and tumor growth in mammary glands of transgenic mice. Both estrogen receptor (ER)-α and ER-β mRNA and protein levels were increased in transgenic mammary glands, and in addition, progesterone receptor (PgR) expression was also upregulated, which suggests that the ER dependent cell growth system and the E2-synthesis mechanism are closely coordinated in mammary tissue. Other experiments using male aromatase transgenic mice or knockout mice showed consistently that aromatase plays a crucial role in mammary development and pre-neoplastic or neoplastic changes. These data confirmed the importance of intragland estrogen synthesis even in menopausal status. Another interesting gene overexpressed in aromatase transgenic mice was vascular endothelial growth factor (VEGF). VEGF is known to exert essential effects in neovascularization, particularly tumor-derived new vessel growth. Although the mechanism of VEGF upregulation by aromatase overexpression is still largely unknown, it is speculated that upregulation of VEGF seems to facilitate hormone-dependent neoplastic
changes in mammary glands by supplying new vessels. Expression of several cell cycle regulatory molecules such as cyclin D1 and cyclin E were remarkably increased in this transgenic model.

Zhou et al. clarified a novel aspect of the interaction between breast tumor cells and breast adipose fibroblasts. According to analyses on transcriptional regulation of aromatase in fibroblastic cells, Zhou et al. proposed an intriguing model in which breast cancer cells control aromatase expression in adipose fibroblasts via the secretion of soluble mediators that selectively downregulate essential adipogenic factors, inhibit the differentiation of fibroblasts to mature adipocytes, and stimulate expression of aromatase in those cells. The mediators seem to be breast cancer–specific because conditioned media from other types of tumor cells did not induce such effects. Enhanced binding of CCAAT/enhancer binding protein β to a promoter II regulatory element in this process was also reported. This model may explain in part why aromatase plays particular roles in malignant breast lesions, and targeting differentiation of fibroblasts to adipocytes may be a novel therapeutic approach.

**Clinical Outcomes of Aromatase Inhibitors**

Outcomes of Phase III clinical trials comparing aromatase inhibitors with megestrol acetate (MA) in the second-line treatment for metastatic breast cancer patients demonstrated that 3rd generation aromatase inhibitors including anastrozole, letrozole and exemestane seem to be at least equally effective as MA in clinical response rate and time to progression (TTP) (Fig 2, 3). In particular, letrozole and exemestane showed a significantly higher response rate and a significantly longer TTP as compared with MA, respectively (Table 1, 2). The clinical trials comparing aromatase inhibitors and tamoxifen in the setting of first-line treatment for metastatic breast cancer patients have been subsequently conducted (Table 3). Mouridsen et al. documented the latest results on the comparison between letrozole (2.5 mg/day, n = 453) and tamoxifen (20 mg/day, n = 454) that letrozole was significantly superior to tamoxifen in overall response rate and time to progression. Response rates of letrozole and tamoxifen were 30% and 20%, respectively. Clinical benefit, including stable disease for more than 6 months, was 49% in letrozole group, whereas that in the tamoxifen group was 38%. Importantly, in this crossover design trial, the letrozole group had a significantly longer TTP compared with the tamoxifen group. Nabholtz et al., and Bonneterre et al. reported the outcomes of a comparative study between anastrozole and tamoxifen, respectively. The former group demonstrated that anastrozole showed