· 综述 ·

New development of breast cancer histological evaluation related to determination of therapeutic option for the patients

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Breast cancer is a heterogenous disease with molecular alterations, cellular composition, and clinical outcome. The more we know about the tumor characteristics underlying the heterogeneity of the disease, the greater the opportunity to refine treatment options. Great emphasis has been placed upon histopathological characteristics of breast carcinoma cells in order to define better treatment options for breast cancer patients[1-2]. The recommendations of St. Gallen 2009 reported eight characteristics which favor the use of chemotherapy, and in particular those that might justify endocrine therapy alone[1]. Four out of the eight characteristics have been defined by histopathological analysis including estrogen receptor (ER) and progesterone receptor (PR) status, histological grade, cell proliferation and peritumoral vascular invasion[1]. The features indicating increased risks of recurrence and thus indirectly supporting the value of adding chemotherapy to endocrine therapy in those patients include lower expression of steroid hormone receptors, grade 3 tumors, high proliferation of carcinoma cells and extensive peritumoral vascular invasion[1]. In addition, St. Gallen 2011 focused on intrinsic subtypes defined by ER, PR, HER-2, histological grade and Ki-67[2-4]. They defined luminal A as ER positive and PR positive, HER-2 negative and 14% or less of Ki-67 labeling index, and luminal B as ER positive and/or high Ki-67 and/or histological grade 3 with or without HER-2 positive[2]. And it was demonstrated that factors recommending for inclusion of chemotherapy included histological grade 3, more than 14% of Ki-67 labeling index, low hormone receptor status, positive HER-2 status and triple negative status[2]. 82.9% of the panels of this meeting agreed that for practical purposes tumor subtypes can be ascertained by non-genetic tests for ER, PR, HER-2 and Ki-67[2], which is and will be quite relevant in the clinical practice at least in Asian countries considering astronomically expensive price tag of these genetic tests. In addition, 75.6% of the panels disagreed the

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choice of therapy depends on tumor subtype as defined by multi-gene array analysis[2], which is also quite reasonable considering the marked value of simple histological analysis. Therefore, it becomes increasingly important that the clinicians are provided with accurate histopathological information in order to base therapeutic decisions. This brief review article summarizes the analysis of histopathological diagnoses of breast carcinoma including ER and PR expressions, HER-2 status, Ki-67 labeling index and other histopathological factors which clinicians should know when seeing the pathology report of the patients.

1 Evaluation of ER and PR

The ER and PR contents of breast carcinomas are important as a prognostic and predictive biomarker, and evaluations of ER and PR status are part of the routine assessment of these neoplasms. As to the method for the detection and quantification of ER and PR, immunohistochemical methods have been preferred because of their relative simplicity, low cost, speed of performance, application to small samples, precise identification of reactive elements, simple methods of fixation and storage, ability to be applied to archival material, and better ability to predict response to adjuvant endocrine therapy owing to validation studies for ER and PR. As for the evaluation of immunohistochemical results, some scoring systems include the Allred score which combines intensity and the number of positive cells to calculate the summative scores[5-7].

Allred score has been one of the most frequently used histopathological techniques to quantify ER and PR expressions[5-6]. In the assessment standard, the proportion score is classified into 6 scales according to the percentage of stained cells, and the intensity score is classified into 4 scales[5-6]. The two scores are combined, and the total score is classified into 0 or 2-8[5-6]. Endocrine therapy is considered likely to be effective when the Allred score is more than 2[6]. Therefore, the results of previous study demonstrated that Allred score was one of the most important factors for deciding whether aromatase inhibitors were indicated, although it was also pointed out that this therapy was still effective in the patients with low Allred scores[6-7].

In Japan, in order to standardize the immuno-histochemical method and to decide on a scoring system, the Japanese Society of Breast Cancer has organized a task force to produce an "adequate evaluation for immunohistochemical evaluation in routine practice for breast cancer", named J-Score[7]. The fundamental concept of the J-Score is that the scoring system only evaluates the number of positive cells without taking the staining intensity into consideration[7]. The assessment scores of

J-Score are as follows: J-Score 0 means no stained cells, J-Score 1 means stained cells ≤1%, J-Score 2 means 1% < stained cells <10%, and J-Score 3 means stained cells ≥10%[7]. The assessment categories have been selected as follows: negative means J-Score 0; uncertain means J-Score 1 and 2; and positive means J-Score 3[7]. However, it awaits further investigations to clarify the clinical relevance of this scoring system.

American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) recommended that the cutoff to distinguish positive from negative cases should be 1% or more ER positive tumor cells[8]. The panels of ASCO/CAP recommended the patients whose breast tumors show at least 1% ER-positive cells should be the candidates for endocrine therapy and withholding endocrine therapy if less than 1%[8]. In addition, the panels of St. Gallen expert consensus meeting also agreed that any positive level of ER expression was considered sufficient to justify the use of endocrine adjuvant in almost all the patients[1]. Therefore, we advocated the optimal cutoff of ER positive ratio as 1%. Endocrine therapy is considered to be effective only when ER is positive in carcinoma cells. However, effectiveness of endocrine therapy is different between the patients with strongly positive expression (more than 10%) and those with weak ones (1% to 10% positivity). Therefore, the results of previous studies have suggested that weak ER expression group may be benefited by administration of concurrent adjuvant or neoadjuvant chemotherapy[1]. We also stressed that it is reasonable for clinicians to discuss the pros and cons of endocrine therapy with patients whose tumors contain low levels of ER by immunohistochemical analysis (1% to 10% weakly positive cells) and to make an informed decision based on the balance. It is also obviously important for clinicians to communicate effectively with pathologists evaluating these ER and PR status of individual patients.

2 HER-2 status

The proto-oncogene HER-2/neu (c-erbB-2) has been localized to chromosome 17q and encodes a transmembrane tyrosine kinase growth factor receptor[9-10]. The name for the HER-2 protein is derived from human epidermal growth factor receptor, as it features substantial homology with the epidermal growth factor receptor (EGFR)[9-10]. Amplification of HER-2 gene has been reported in 20%–35% of primary breast cancers, and the results of earlier studies also suggested that an amplification of the gene is an indicator of poor prognosis in breast cancer patients[9-10]. HER-2 status is predictive for several systemic therapies[11-12]. Retrospectively obtained results from prospectively conducted randomized clinical

trials appear more definitive in suggesting that HER-2 positivity is associated with response to anthracycline therapy; although, this effect may be secondary to co-amplification of HER-2 with topoisomerase Ⅱ, which is a direct target of these agents[11]. In addition, the other study also suggested that HER-2 may predict the eventual response and benefits from paclitaxel in either the metastatic or adjuvant setting[12]. Results of previous studies demonstrated that agents that target HER-2 are remarkably effective in both the metastatic and adjuvant settings[13]. Trastuzumab (Herceptin; Genentech, South San Francisco, CA), a humanized monoclonal antibody, improves response rates, time to progression, and even survival when used alone or added to chemotherapy in metastatic breast cancer[13].

It is therefore important for clinicians to be provided with accurate HER-2 information on which to base therapeutic decision of anti-HER-2 therapy. A Japanese ring study for HER-2 testing recommended that the specimens were fixed with 10% formalin for 6 to 48 hours and embedded in paraffin blocks[14]. Tissue sections, 4 μm thick for immunohistochemical analysis and 5 μm thick for fluorescence in situ hybridization (FISH) were mounted on silane-coated slides[14]. The ASCO/CAP recommended that a positive HER-2 test is defined as either immunohistochemistry result of 3 + cell surface protein expression, defined as uniform intense membrane staining of >30% of invasive tumor cells, or FISH result of amplified HER-2 gene copy number, average of more than 6 copies/nucleus for test systems without internal control probe, or HER-2/CEP17 ratio of more than 2.2, where CEP17 is a centromeric probe for chromosome 17 on which the HER-2 gene resides[15]. An equivocal result (2 +) for immunohistochemistry is complete membrane staining that is either non-uniform or weak in intensity but with obvious circumferential distribution in at least 10% of cells[15]. Some but not all of these samples may have HER-2 gene amplification and require additional testing to define the true HER-2 status[15]. The equivocal range for FISH assays is defined as HER-2/CEP17 ratios from 1.8 to 2.2 or average gene copy numbers between 4.0 and 6.0 for those systems without an internal control probe[15]. However patients with a HER-2/CEP17 ratio between 2.0 and 2.2 were formerly considered HER-2 positive and were eligible for treatment in the adjuvant trastuzumab trials[15]. Therefore, available efficacy data do not support their exclusion from the therapy with trastuzumab[15], although it awaits further investigations for clarification.

3 Ki-67 labeling index

Because rapid tumor proliferation is a critical feature for tumor aggressiveness, proliferation markers have been extensively evaluated as prognostic tools in human

malignancies. Ki-67 is a cell-proliferation-associated antigen that is expressed in all stages of the cell cycle except G0 or the resting phase of the cell cycles[16]. Determination of the percentage of Ki-67 expression has become a standard method to assess the proliferative activity of tumor cells[16]. The nuclear protein Ki-67, present in cycling cells, is an indicator of tumor proliferation and has been found to be a prognostic marker in breast cancer[17-18]. High Ki-67 labeling index is reportedly predictive of responsiveness to preoperative chemotherapy[17-18]. St. Gallen 2009 recommended that Ki-67 labeling index were considered important for determination of prognosis, and importantly to indicate the potential value of the addition of chemotherapy to patients with receptor-positive disease as further validation of findings in this regard was felt to be necessary[1]. It also recommended conventional measures of proliferation include assessment of Ki-67 labeling index, eg, low: ≤ 15%; intermediate: 16%-30%; high: > 30%, and pathological description of the frequency of mitoses[1]. In addition, St. Gallen 2011 also recommended that luminal B was defined as ER positive, PR negative and/or high Ki-67 which was more than 14% and/or histological grade 3 with or without HER-2 positive[2]. They recommended that one of the factors for an inclusion of adjuvant chemotherapy was more than 14% of Ki-67 labeling index[2]. Immunostaining of Ki-67 is becoming a routine requirement for invasive breast cancer in clinical practice. The examination and staining methods of Ki-67 vary in different geographic settings. Therefore, it is necessary that the guidelines for staining, evaluation methods and standardization should be established in near future.

4 Other histopathological factors

Angiogenesis plays a pivotal role not only in human normal development but also in pathophysiological conditions such as inflammation and carcinogenesis[19]. We evaluated Vasohibin as a newly identified biomarker of angiogenesis[19]. Vasohibin is a recently identified negative feedback inhibitor or suppressor of angiogenesis induced by VEGF[19]. Our previous study demonstrated that the Vasohibin immunodensity was significantly higher in invasive ductal carcinoma than in non-invasive ductal carcinoma[19]. In addition, the results of double immunostaining analysis which can simultaneously demonstrate two different proteins in the same cells, demonstrated the significant positive correlation between Ki-67 positive proliferating vascular endothelial cells, which may represent neovascular formation, and Vasohibin positive endothelial cells[19]. Therefore, Vasohibin is considered a more appropriate biomarker for intratumoral neovasculari-zation compared to CD31[18].

Triple-negative breast cancer is generally considered to be associated with aggressive clinical behavior partly due to the limitations of the specific therapies currently available in clinical practice[20]. However, it is also true that a marked heterogeneity exists in terms of clinical outcome or prognosis and response to various chemotherapeutic agents among triple-negative breast cancer patients[20]. Miyashita et al[20] demonstrated that triple-negative breast cancer could be further subscribed into three different groups according to the risk score system evaluating the following five prognostic variables: pathological tumor size, pathological node status, basal-like type, Ki-67 labeling index and neovascu-larization. Such a classification, which can be performed in diagnostic pathology laboratory, can be useful as a decision-making tool for triple negative patients[20].

Several biomarkers obtained by immunohisto-chemical evaluation are in general considered useful for differential diagnoses in breast lesions whether the lesions are benign or malignant and whether the carcinomatous lesions are invasive or non-invasive. It is important that the clinicians are provided with accurate histopathological informations, also it is equally important that which informations are more important among various histopathological factors of the patients provided by the pathologists. We mainly determined the treatment for breast cancer patients according to the biomarkers of ER, HER-2 and Ki-67[1-2]. Therefore, it is considered very important at least at this juncture for the clinicians managing the patients with breast cancer to pay particular attentions to the status of ER, HER-2 and Ki-67 among many histopathological factors provided by the pathologists. When two patients have similar ER and PR expressions, HER-2 status, Ki-67 labeling index and other relevant histopathological factors, the standardized treatment for special histological types of breast cancer is controversial[1-2]. St. Gallen recommendations reported that endocrine-responsive types such as tubular and cribriform carcinomas may be managed without adjuvant chemotherapy or with endocrine therapy alone. In addition, it is also reported that rare variants such as lobular carcinomas and apocrine carcinomas may require postoperative treatment according to their biological features mentioned above in a manner analogous to that used for ductal carcinoma. However, it is also true that no evidence-based approach to the postoperative treatment of these patients with special histological types has been established. Therefore, we employed the same treatment strategy according to St. Gallen recommendations for invasive ductal carcinoma[1-2]. A previous study demonstrated that to decide the malignant potential of intraductal proliferative lesions, analysis for the staining pattern of cytokeratins was a good reference[21]. Most ductal carcinoma in situ cases were

diffusely positive for luminal cell markers (CK8, CK18 and CK19), but negative for basal cell markers (CK5/6 and CK14)[21]. However, useful ductal hyperplasia showed the mosaic stained patterns for any of these markers, which indicated heterogeneous cell population in benign lesions[21]. Myoepithelial markers including alpha-SMA, myosin calponin, p63 and CD10 were almost consistently positive for benign papillomas but they did not completely distinguish intraductal papillary carcinomas[21]. Preservation of myoepithelial layer was the diagnostic key when looking at benign sclerosing lesions, including carcinoma with pseudo-invasive structures[21].

A fundamental aspect of histopathology has been the recognition that the morphological appearances of tumors can be correlated with the degree of malignancies. As the range of options for the treatment of patients with breast cancer widens, it is the most important that the clinicians are provided with accurate histopathological informations on which therapeutic decisions are based.

【**Keywords**】 breast neoplasms; histological evaluation; therapy

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