Review

Pathological Evaluation of HER2 Overexpression: For the Treatment of Metastatic Breast Cancers by Humanized Anti-HER2 Monoclonal Antibody (Trastuzumab)

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For the treatment of patients with metastatic breast cancer by humanized anti-human epidermal growth factor receptor type 2 (HER2) antibody (trastuzumab), it is important to evaluate HER2 status adequately. This article overviews devel-

opments of basic and clinical research for HER2 gene, and introduces general agreement with the evaluation system of HER2 overexpression with several commentaries from the aspect of histochemistry.

Key words: Breast cancer, HER2, Trastuzumab, Immunohistochemistry, FISH

I. Introduction

In the past three decades, HER2 gene overexpression has attracted many basic and clinical researchers leading to an accumulation of the knowledge and techniques for practical use in the clinical field. HER2 protein is a transmembrane tyrosine kinase receptor for an unknown growth factor. HER2 overexpression is a poorer prognostic factor in breast cancers [26, 28] and a predicting factor for therapeutic responses [2, 8, 15, 18, 30, 37]. The HER2 receptor has also been a target of an anti-receptor agent developed recently. This commercially available humanized anti-HER2 monoclonal antibody (trastuzumab, Herceptin®; Genentech Inc./Roche Inc.) has been administered to patients with HER2 positive metastatic breast cancer.

Histochemical examinations were initially applied as a research tool to study HER2 overexpression, and have become a critical step in the patient therapy. It is essential to evaluate HER2 status adequately in order to select the patients expected to benefit from trastuzumab treatment. In this article, development of basic knowledge of HER2 gene and clinical applications will be reviewed concerning the

relation with applied histochemical techniques.

II. HER2

The *neu* gene was identified by transfection study of DNA of nitrosoethylurea-induced rat neuroblastomas [24, 27]. Other groups isolated human *erbB*-related genes HER2 [6] and *c-erbB-2* [25] independently. Subsequent sequence analysis and chromosomal mapping studies revealed these three genes to be identical [6, 25, 36]. HER2/*c-erbB-2* gene is located on chromosome 17q21 [7, 23], and the HER2 product is a transmembrane tyrosine kinase consisting of an extracellular domain, transmembrane domain and intracellular domain. There are many ligands binding to HER1, HER3 and HER4. EGF, TGF- α , Epiregulin, β -cellulin, HB-EGF and amphiregulin bind to HER1, Neuregulin 1 (NRG1) binds to HER3, and HB-EGF and NRG1-4 bind to HER4 [12]. However, no ligand has been found to bind to HER2 with a high affinity. Ligand-binding to HER1, HER3 and HER4 stimulates homodimer and/or heterodimer formation of these receptors as well as HER2. The intracellular domain is autophosphorylated resulting in intrinsic kinase activity, except for HER3 [9]. Thus, although HER2 and HER3 do not have the ability to moderate signaling alone, heterodimers including HER2-HER3 provide the means for transformation [1, 35], mitogenic activity [21], and cell motility [4] via mitogen activated protein kinase (MAPK), the c-Jun N-terminal kinase (JUNK) pathway [10], and the

Abbreviations: HER2, human epidermal growth factor receptor type 2; FISH, fluorescence *in situ* hybridization; IHC, Immunohistochemistry.

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Akt pathway [38]. Slamon *et al.* [28] reported in 1987 that breast cancers with HER2 overexpression were associated with larger numbers of lymph node metastases and poorer prognosis. Since then, these have been many reports that erbB-2 overexpression predicts poorer prognosis in breast cancer patients [26]. The predictive value of HER2 overexpression for therapeutic response has been reported as well. Breast cancers overexpressing HER2 respond well to doxorubicin [18, 30], and resist hormone therapy (Tamoxifen) [15, 37] and polychemotherapy (cyclophosphamid (CPA), methotrexate and 5-fluorouracil; CMF) [2, 8].

III. Trastuzumab

Trastuzumab is produced by genetic recombination. Five percent of trastuzumab is derived from the mouse monoclonal antibody (4D5) at the region recognizing HER2 and 95% is derived from human IgG₁. Host immune reaction to trastuzumab is thus minimized and the antigen-binding activity of HER2 is retained in the Fab region. Studies in tumor xenografts confirmed that murine Mab 4D5 inhibits growth of human breast cancer cells overexpressing HER2 [16] and a humanized monoclonal antibody (trastuzumab) was produced [3]. Preclinical studies [20, 31] showed that trastuzumab has the same or greater anti-tumor potency, and it induces antibody-dependent cell-mediated cytotoxicity (ADCC) against human tumor cell lines. A phase III clinical trial demonstrated that the overall response rate to trastuzumab was 15% [5] as a single agent after chemotherapy. Administration of trastuzumab in addition to chemotherapy provided a longer time to progression, higher rate of objective response, longer survival and other clinical benefits [29].

IV. Evaluation of HER2 Overexpression

Evidence based medicine

As the concept of “evidence based medicine” has been accepted in the clinical field, the treatment has been selected based on results of studies performed by the large number of patients but not only by individuals’ experiences. Evaluation of HER2 status is also in this current. HER2 gene amplification was evaluated by Southern blotting in early studies [28] followed by IHC using CB11 or 4D5 monoclonal antibodies (clinical trial assay; CTA) in clinical trials [5, 29]. In these studies, expression was estimated by scoring system as 0, 1+, 2+, 3+ using standardized criteria, and the patients with IHC 2+/3+ were listed for trastuzumab treatment. Recently, it has been emphasized that FISH is a more reliable method by the effective outcome (41%) of trastuzumab treatment compared to IHC staining of 2+/3+ (26%) [33, 34], although the patient group showing IHC 3+ (35%) was treated at a similar response rate with trastuzumab [34]. The consensus at the present time is, therefore, that patients with metastatic breast cancer interpreted as 3+ by IHC are appropriate for trastuzumab treatment, while patients with a score of 0 or 1+ are not appropriate. Treatment of patients

with breast cancers scored as 2+ will be decided depending on several factors including patients’ will, clinicians’ opinion or other procedures like FISH at the present time.

From the aspect of histochemistry

Concordant rate between IHC and FISH

The concordant rate between CTA and FISH was 82% overall, and 89% between IHC 3+ and FISH [17]. It is generally agreed that there are highly concordant rates between HER2 amplification detected by FISH and HER2 overexpression interpreted as 3+ by IHC [11, 17, 22]. For breast cancers evaluated as 2+, consistent results between IHC and FISH were not obtained (27%) [17].

Advantages and disadvantages of IHC and FISH

The immunohistochemical method has the advantages of lower cost, easier techniques and a shorter time for evaluation in practical routine pathology. It has been reported that the different antibodies available for HER2 protein yielded varying overexpression rates, from 17.2% to 42% [13, 14, 19]. This variation is a major disadvantage of immunohistochemical methods. FISH is a more reliable method, but requires a more involved process, higher technical skill, higher cost and more effort to reliably evaluate. The treatment and conditions of the samples are critical for the FISH method. The kinds of fixatives, duration until fixation and period of fixation are essential for reliable results.

Evaluation of HER2 protein by IHC

Immunohistochemically HER2 protein is evaluated by population of positive cells and strength of staining (Table 1). When positive cells are identified in more than 10% of the population, HER2 status is evaluated as 1+, 2+, 3+ depending on the staining strength. The consensus in the histochemical field is that strength of staining does not always correlate to the amounts of protein production, and therefore one should not evaluate the staining strength. However, it has been accepted that staining strength is well correlated to HER2 gene amplification as described above.

There are many antibodies recognizing extracellular and intracellular domain of HER2 proteins. These include TAB250 (Zymed, BioGenex) and SV2-61γ (Nichirei) for extracellular domain, and CB11 (Ventana, Biozac, Novocas-

Table 1. Staining pattern for evaluation of HER2 protein**

Score	Staining Pattern
0	no staining or membrane staining is detected in less than 10% of tumor cells
1+	faint or barely positive staining is detected in more than 10%
2+	moderate and/or incompletely circumscribed membrane staining is detected in more than 10%
3+	strong and completely circumscribed membrane staining is detected in more than 10%

** cytoplasmic staining is ignored.

Fig. 1. Immunohistochemical features of HER2 protein. Almost all of the carcinoma cells strongly express HER2 protein at the cell border. The case was evaluated as “3+” (IHC for HER2, HercepTest).

tra, BioGenex), HercepTest (DAKO), polyclonal antibodies (Nichirei) for intracellular domain. The polyclonal antibody (DAKO) showed higher positive results and more false positive cases than CB11 or TAB250 [32].

Standardization for practical use

For the application of histochemical method to practical use, there are several matters that should be considered. Selection of method and evaluation will lead to inter-institutional and inter-observer diversities that affect the patient benefits. Commercially available staining kits may provide standardization of the staining system. A staining kit is available for IHC (HercepTest, DAKO Carpinteria, CA) (Fig. 1), and two commercially available kits are available for FISH (PathVysion™ (Vysis) and INFORM™ (Ventana)) in the United States. An atlas and a guideline produced from the trastuzumab distributing company (www.her2.org/) may be helpful for standardized evaluation. In other countries, development of quality insurance systems for immunohistochemistry in England and United States are attractive. It has been developed initially for immunohistochemical examinations of hormone receptors (estrogen receptor, progesterone receptor). These central laboratories published recommendations for adequate fixation and treatment of the sections, procedures for IHC, and appropriate evaluation system, which is a possibility for global standardization.

V. Summary

In this article, general agreement on application of IHC and FISH for the patients with metastatic breast cancers and the situations around HER2 gene overexpression are introduced.

VI. References

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