Review

Molecular Analysis and Therapeutic Application; Molecular Targeting for Breast Cancer Treatment

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Received April 5, 2000; accepted June 2, 2000

Advanced biotechnologies have provided benefits to detect a variety of cellular molecules for tumor diagnosis, determination of malignancy, and prognosis of cancers. Improved understanding of the molecular basis of cancers will lead to molecular targeted approaches to cancer prevention and treatment. Monoclonal antibody-based therapeutics targeting cell surface receptor such as HER2/*neu*/c-erbB2 oncoprotein on breast cancer cells has shown efficacy in clinical trials. HER2 pro-oncogene is a member of the epidermal growth factor receptor family, and HER2 oncoprotein is a well-characterized predictor of tumor aggressiveness. The overexpression of HER2 has been found in about 25% to 30% of breast cancers and associated with poor prognosis, resistance

to hormonal therapy and lack of sensitivity to some adjuvant chemotherapy. The recombinant anti-HER2 monoclonal antibody, trastuzumab (Herceptin), was evaluated in clinical trials for treatment of HER2-overexpressing metastatic breast cancers. The administration of the trastuzumab has produced an objective response in patients with metastatic breast cancers, suggesting that the trastuzumab will be a new molecular targeting therapeutic agent for patients with HER2-overexpressing cancer. Thus, a test result of HER2 overexpression will be a valid criterion in determining eligibility for the trastuzumab therapy, and reliable detection of HER2 overexpression is an important prerequisite for the success of the trastuzumab therapy.

Key words: HER2/*neu*/c-erbB2, Breast cancer, Trastuzumab, HER2 overexpression

I. Introduction

Progressive molecular biotechnologies used for histochemistry and cytochemistry have been providing a variety of benefits to identify specific proteins, peptides, and nucleic acids in normal and abnormal cells. Molecular analyses upon tumorigenesis, cell growth/apoptosis, and metastasis/invasion of malignant cells have been enthusiastically accomplished, and the accumulated data from the analyses are now available for tumor diagnosis, determination of the degree of tumor malignancy, and prognosis of malignant tumors. Based on the discovery of oncogenes and tumor-suppressor genes, the idea that

cancer is a genetic disorder has been conceptualized [9, 21]. The oncogenes are activated through their DNA mutation, rearrangement, or amplification, and then these genetic disorders induce abnormal cell proliferation and promote cellular tumorigenesis.

Improved understanding of the molecular basis of cancers has resulted in the identification of molecular genetic abnormalities and the consequences of such abnormalities, the identification of growth factors and their specific receptors, and the characterization of the intracellular signal transduction pathway. Expansion of the understanding of oncogenic signal transduction pathways will lead to molecular targeted approaches to cancer prevention and treatment. Many of the oncogenic molecules may be used as a target to block the signaling pathway. Recently, one of the human epidermal growth factor receptors: HER2, which transmits growth signals

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to the nucleus, stimulates growth, and in some cases leads to oncogenic transformation, has become a specific target for a monoclonal antibody-based therapeutic application of HER2-overexpressing breast cancers [2, 8]. Therefore, a well-standardized bioassay to determine HER2 overexpression is an important prerequisite for the success of therapy.

II. A Prognostic and Predictive Factor: HER2/*neu*/c-erbB2

Immunohistochemistry is the most useful tool in diagnostic histopathology. The applications of the diagnostic immunohistochemistry mainly comprise the detection of differentiation antigens, tissue specific antigens, and the degree of malignancy in human cancers. Additionally, tumor biological factors such as hormone receptors and epidermal growth factor receptors associated with the prognosis of cancers and therapy decisions have been evaluated [6, 11]. The epidermal growth factor (EGF) family of receptor tyrosine kinase is a very important mediator of cell growth, differentiation, and survival [3]. HER2/*neu*/c-erbB2 pro-oncogene is a member of the EGF receptor family; its oncoprotein is a well-characterized predictor of human tumor aggressiveness [17, 18]. The HER2 gene amplification results from dysregulation of the HER2 gene, and leads to the consequence of HER2 overexpression on the tumor cell surface.

The HER2 gene amplification and overexpression of the HER2 oncoprotein have been studied in a wide variety of human malignant tumors in breast, ovary, lung, stomach, and oral cavity by means of FISH (fluorescence *in situ* hybridization) and immunohistochemistry (IHC) [7, 19, 22–24]. The overexpression of the HER2 has been found in about 25% to 30% of breast cancers and associated with poor prognosis, resistance to hormonal therapy and lack of sensitivity to some adjuvant chemotherapy [4, 16–18]. The majority of breast cancer patients with metastasis have been treated by chemotherapy and hormonal therapy, and the measurement of hormone receptor, estrogen receptor (ER) and progesterone receptor (PR), has an important value as a predictor of response to hormone therapy. With respect to the HER2, its overexpression may serve as a poor prognostic marker, but there was not sufficient value for predicting response to a given therapy [5, 13].

III. A Target Molecule for Cancer Therapy: HER2/*neu*/c-erbB2

The exciting results demonstrating that a HER2 targeting agent, trastuzumab (Herceptin; Genentech, San Francisco, CA) has efficacy in breast cancer have renewed interest in the overexpression of the HER2 oncoprotein [19]. Several monoclonal antibodies directed against HER2 inhibited the proliferation of monolayer cultures of breast cancer cells overexpressing HER2, and the most

potent growth inhibitory antibody was 4D5 [8]. The 4D5 monoclonal antibody has been investigated particularly *in vitro* and in xenograft models of human breast cancer that overexpress HER2 oncoprotein [1]. In general, monoclonal antibodies are capable of binding to a wide variety of potential targets, and it has been hypothesized that this particular property can be harnessed with therapeutic benefit. Nevertheless, the majority of early monoclonal antibody-based therapeutics have not presented sufficient outcomes since the administration of monoclonal antibodies derived from mouse stimulated host immune response against the foreign proteins [12].

However, monoclonal antibody-based therapeutics is now envisioned as therapeutic targeting agents with the advanced DNA recombinant technologies that are capable of manipulating the structure of antibodies. In order to reduce human anti-mouse antibodies (HAMA) generation by human immune system, the 4D5 monoclonal antibody has been humanized by transplanting the complementarily determining regions (CDRs) of the 4D5 to a human framework [2]. The recombinant humanized 4D5 monoclonal antibody, trastuzumab (Herceptin), was evaluated in human clinical trials for treatment of HER2-overexpressing metastatic breast cancers [20]. The administration of trastuzumab has produced an objective response in patients with HER2-overexpressing metastatic breast cancers, suggesting that trastuzumab will be a new molecular targeting therapeutic agent for patients with HER2-overexpressing cancers.

IV. Assays for Determination of HER2/*neu*/c-erbB2 Expression

Reliable detection of HER2 overexpression is an important prerequisite for the success of trastuzumab therapy. There are several methods available to measure DNA, RNA or protein of HER2 expression, but they differ in the practicality and reproducibility. For routine evaluation of HER2 status, IHC and FISH appears useful, but the methods for HER2 measurement need to be well optimized and standardized [15]. There are several practical reasons why FISH is not widespread in many pathology laboratories to determine HER2 status. The FISH assay requires fluorescence microscopy and a time-consuming process to interpret HER2 gene amplification. The most widely used assay for the assessment of HER2 status is IHC, which measures overexpression of the HER2 oncoprotein. The immunohistochemical determination of HER2 status can be easily performed on routinely formalin-fixed, paraffin-embedded specimens, and the techniques required for IHC assay is more familiar, less expensive, and simpler compared with FISH.

However, in order to judge overexpression of the HER2 immunohistochemically, the IHC assay may have to be designed as a semi-quantitative immunohistochemical assay. The validity of immunostaining results of HER2 expression depends upon tissue fixation,

unmasking of epitopes of the HER-2, and different primary antibodies against HER2, as well as systems for scoring and assessment of the staining [10, 13]. Therefore, it is critical that any variability in scoring and agreement between different laboratories rely on a

standardized HER2 determination across laboratories. A scoring system to assess the degree of HER2 staining shown as Fig. 1 may be proposed to provide a valid criterion in determining eligibility for trastuzumab therapy.

Fig. 1. Immunohistochemical staining and interpretation of HER-2 overexpression. No staining at all: score 0+ (A), a faint perceptible membrane staining: score 1+ (B), a weak to moderate staining of the entire membrane: score 2+ (C), and a strong staining of the entire membrane: score 3+ (D). The (C) and (D) are interpreted as positive cases for HER-2 overexpression, while the (A) and (B) are judged as negative cases.

V. References

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