

HER 2 As A Potential Target For Cancer Therapy

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Abstract

Growth factor and their receptors are playing promising roles in cell development, growth and differentiation. Many receptor possess intrinsic tyrosine kinase activity that is activated upon the receptor's interaction with its ligand. Abnormal expression of human epidermal growth factor receptor 2 (HER2) is frequently expressed and observed in a cell membrane of number of primary tumors may contribute to transformation and tumorigenesis. HER2 is also called c-erbB-2, HER2/neu, and human EGF receptor 2. High-level activation of the HER 2 kinase results in the activation of downstream signaling molecules, such as the MAP kinases and the PI 3/ Akt kinases, which both drive proliferation and inhibit apoptosis of the cells.

Keywords: HER2, Tyrosine kinases, Epidermal growth factor (EGFR), MAP kinases

1. Introduction

Her-2/neu, also called Human Epidermal Growth Factor Receptor-2, is a cellular receptor that resides in the cell membrane itself. It actually spans the membrane and protrudes into the cell (called the intracellular domain) on one side and out of the cell on the other side (the extracellular domain). A variety of proteins found in the blood pass by the receptor, some may recognize it, and a smaller fraction will bind to it. Binding to the extracellular domain of the transmembrane receptor initiates a variety of signals within the cell including those commanding cell division, growth, and differentiation. These signals are only transmitted, however, once a protein has bound to its complementary receptor on the cell surface¹.

Role of Her -2-Neu protein

Her-2/neu, also called Human Epidermal Growth Factor Receptor-2, is a cellular receptor. A receptor is a protein that resides in the cell membrane itself. It actually spans the membrane and protrudes into the cell (called the

intracellular domain) on one side and out of the cell on the other side (the extracellular domain). A variety of proteins found in the blood pass by the receptor, some may recognize it, and a smaller fraction will bind to it. Binding to the extracellular domain of the transmembrane receptor initiates a variety of signals within the cell including those commanding cell division, growth, and differentiation. These signals are only transmitted, however, once a protein has bound to its complementary receptor on the cell surface. The protein in the blood stream that binds and activates the Her-2/neu receptor is called Human Epidermal Growth Factor. When binding occurs, specific signals such as cell division, growth, transformation and differentiation, are transmitted from the extracellular domain inside the cell. This is part of a normal physiologic process. However, some cells can have too many Her-2/neu receptor proteins on their cell surface, and thus may receive too many signals. The multiple signals may result in aberrant cell function and even cancer.^{2,3}

I. HER 2 structures and function:

Her2 is a proto-oncogene that encodes an 185 kDa transmembrane receptor tyrosine kinase which exhibits extensive homology to epidermal growth factor (EGFR)⁴. Her2 is also known as the neu and cerbB2 proto oncogene. The p185 her2 glycoprotein consists of an extracellular domain (ECD,p105)⁵, a single transmembrane segment and a cytoplasmic tyrosine kinase domain. Despite the extensive homology to the EGFR, no single ligand that binds HER2 with high affinity has been yet identified^{6,7}. HER2 tyrosine kinase seems to have basal activity in the apparent absence of a ligand and this activity is significantly enhanced by ligand induced heterodimerization of the receptor. Evidence has accumulated that receptor dimerization is essential for receptor activation. The HER2/HER3 dimer is the most active in mammals; the HER2 receptor plays an important role in the development of cardiac and neural tissue. Scientist observed that mice carrying the HER2 null allele were unable to survive owing to lack of cardiac trabeculae and neural tissue underdevelopment. The HER2 oncogene can be

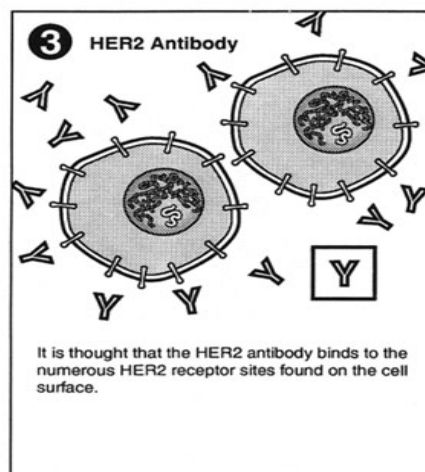
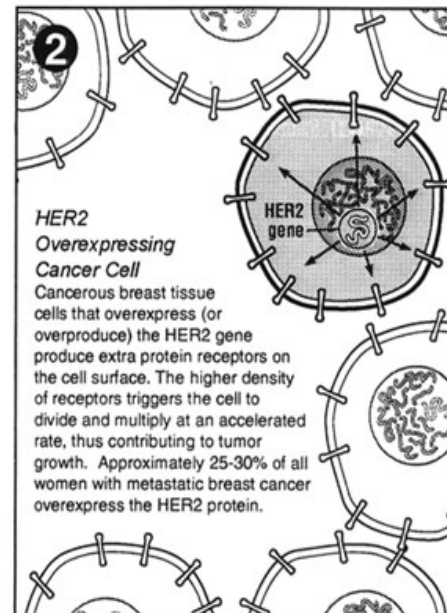
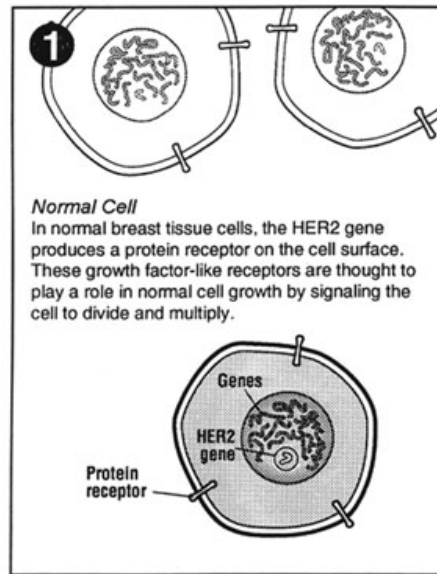
activated by point mutation, gene amplification and/or over expression⁸.

II. HER2 and cancer:

Growth factor and their receptors are known to play critical roles in cell development, growth and differentiation. Many receptor posses intrinsic tyrosine kinase activity that is activated upon the receptor's interaction with its ligand. Abnormal expression of human epidermal growth factor receptor 2 (HER2) is frequently observed in a number of primary tumors, suggests that the overexpression of this growth factor receptor may contribute to transformation and tumorigenesis. In most cases, HER 2 protein overexpression is the result of gene amplification, and overexpression has been correlated with poor clinical outcome in patients with breast and ovarian cancer. Laboratory data have demonstrated that overexpression of HER 2 is sufficient to stimulate the tyrosine kinase activity of this receptor. Furthermore, high-level activation of the HER 2 kinase results in the activation of downstream signaling molecules, such as the MAP kinases and the PI 3/ Akt kinases, which both drive proliferation and inhibit apoptosis of the cells^{9,10}.

Approximately 25% to 30% of patients with breast and ovarian cancer overexpress HER 2. Similar association may exist for lung adenocarcinoma and gastric cancers. These data encourage the exploitation of HER 2 as a potential target for cancer therapy.

The receptor protein Her-2/neu is encoded by the DNA found on the c-erb B-2 gene. Each cell is supposed to have two copies of a single gene (one inherited from each maternal and paternal inheritance). For unclear reasons, some cancer cells have multiple copies of the c-erb B-2 gene. This is known as gene amplification. Multiple copies of the gene lead to too many proteins being produced. This is known as protein overexpression. The significance of protein over production is still not entirely clear but it has become a marker for certain cancers. For example, it was noted that about one fifth to one third (20%-30%) of all breast cancers over produce this protein. Much research has been done to try to understand how these tumors may be different from other breast cancers. There are ongoing studies for Her-2/neu in disease prognosis, predicting response to treatment, and estimating disease recurrence. Her-2/neu positive breast cancers tend to be more aggressive in that they grow more quickly, may be resistant to some available chemotherapies, and are more likely to recur following treatment^{11,12}.



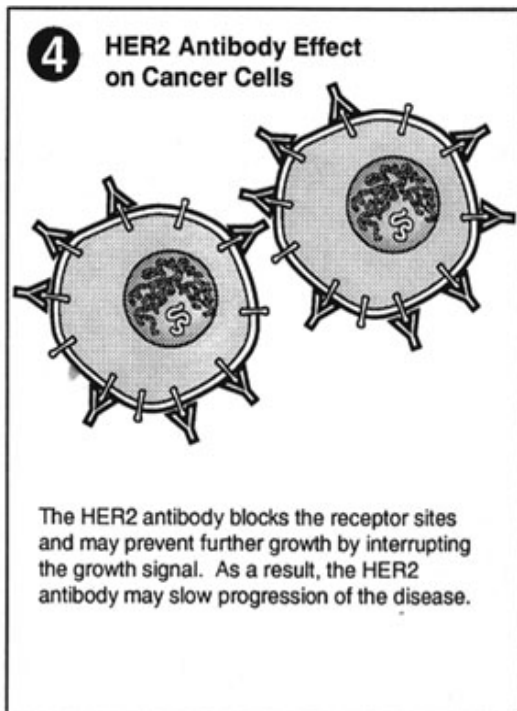


Fig. 1: Her-2-neu antibody and cancer cells¹³iii. Methods to study Her-2/neu overexpression

In order to diagnose Her-2/neu overexpression or c-erb B2 gene amplification, actual breast cancer tissue is required. When patients undergo a biopsy, the tissue is sent to the pathologist. In addition to histology (looking under the microscope to see what the cells actually look like), several additional tests are routinely completed. Testing for Her-2/neu overexpression has become the standard of care for breast cancer. (Table 1)^{14, 15, 16}

1. Immunohistochemistry method

Immunohistochemistry (IHC) is the most widely available and least expensive test for Her-2/neu overexpression.^{13,17} The laboratory uses an antibody that recognizes the Her-2/neu cell surface receptor. These antibodies are specifically manufactured so that one end recognizes and binds to the receptor protein and the other end is labeled with a color or a dye that is easily detected under the microscope. The antibody is allowed to mix and bind with the cells for some time. The bound antibody will stay adherent to the tissue sample, but the unbound or excess antibody will be washed off. The pathologist can then look under the microscope and evaluate the intensity of the color or dye as an indication of the amount of Her-2/neu present on the cell surface.¹⁵ An IHC test is reported as a score of 0, 1+, 2+, and 3+. A score of 0 or 1+ implies very little bound antibody and therefore,

likely very small amount of Her-2/neu. A score of 3+ is considered strongly positive with Her-2/neu overexpression. A score of 2+ is thought to be weakly positive, and, some studies indicate, may require further evaluation or testing.^{18,19,20,21}

Table 1: Methods of assessing HER-2 status in breast cancer

Method	Advantages	Disadvantages	Clinical use
Western blot	Widely available; Relatively inexpensive	Semiquantitative; Ab variability; tumor extract is required	Not in clinical use.
PCR	Rapid; specific; sensitive; small amount of starting material	Semiquantitative	Not in clinical use.
IHC	Widely available; relatively inexpensive	Semiquantitative; Ab variability; subjective interpretation	FDA-approved; most frequently used clinically
FISH	Specific; quantitative; strong correlation with response to trastuzumab	Expensive; requires specialized equipment not widely available	FDA-approved; valuable for confirmation of HER-2 status if IHC score is 2+
ECD ELISA	Serum easily obtained	ECD levels do not always correlate with tumor load	FDA-approved to monitor response to chemotherapy; multicenter prospective study ongoing in patients on trastuzumab
CISH	Useful, Simple, reproducible and less expensive	The current CISH procedure is based on a single color detection of one probe and does not allow correction for HER-2/neu amplification	Should be considered a Viable alternative to FISH analysis for selecting IHC 2+ scored patients for Trastuzumab therapy
Tissue microarray	Allows the analysis of hundreds of tumor samples simultaneously	Extremely small tissue samples taken from the original tissue may not always be representative of the entire tumor	Highly reliable method for analyzing Her-2 oncogene amplification by FISH.

2. Fluorescence in situ hybridization method

Fluorescence in situ hybridization, or FISH, is a more expensive and technically more difficult test that is also used to ascertain Her-2/neu overexpression and is often used to confirm 2+ IHC score. The FISH test has the advantage of looking at each tumor cell individually. Probes tagged with fluorescent labels are

used to identify the *c-erb B2* genes themselves. If more than two fluorescent lights per cell are noted, then the cell is thought to over express Her-2/neu. Agreement among FISH laboratories is more reproducible; its disadvantage is that the expensive technology required is not often available in smaller clinics and rural hospitals.^{22,23}

3. ELISA method

In advanced disease, there may be Her-2/neu proteins in the blood. Blood tests may be done to try to discern the level of Her-2/neu. Rising levels may indicate disease progression, while a falling level may mean disease regression.

4. PCR method

There are other tests used to diagnose Her 2/neu expression, but they are largely experimental. The PCR test, for example, has received much attention lately. Although it is considered to be a more sensitive test, it is difficult to interpret. One needs to measure the Her-2/neu expression on the actual cancer tissue, not on adjacent normal tissue. When doing IHC or FISH, the pathologist can look under the microscope and see which cell is cancerous and which is not. The PCR test does not use whole cells, rather just the DNA from those cells. Thus, it is impossible to see if the DNA came from a cancer cell or a normal cell.²⁴

Conclusion

HER2 has long been known among pathologists and oncologists for its potential role as a tumor and prognostic marker. This protein exists on the surface of epithelial cells and functions in the normal cell as a receptor for a cellular growth factor. Upon binding of extracellular ligands or overexpression, HER-2/neu becomes phosphorylated and mediates activation of the growth-promoting Ras/MAP kinase cascade, as well as the phosphatidylinositol-3 kinase and phospholipase C gamma pathways. Hyperactivation of all these signaling pathways results in an enhanced and, often uncontrolled, cell growth. Consistently, human cancer cells engineered to express high levels of HER-2/neu, with constitutive activation of the receptor, showed a higher tumorigenicity in vivo than parental cells, leading to a significantly reduced survival of grafted animals. HER-2/neu testing, providing a very useful marker in clinical decision making, was also "falling victim" to a high degree of variability in detection and reporting and leading, at times, to inaccurate or nonreproducible classification of patients as HER-2/neu-positive or -negative. In view of the

critical importance of such classification for diagnostic and prognostic purposes, standardization of HER-2/neu testing and reporting has become mandatory.

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