Overexpression/amplification of Her-2/neu in malignant tumors-A Short Review

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Abstract

Her-2/neu overexpression has been shown to play a significant role in development and progression of many malignant tumors of body, mostly affects tumors of epithelial origin. Her-2 is a protein involved in normal cell growth. However, HER2/neu may be made in larger than normal amounts by some types of cancer cells.This may cause cancer cells to grow more quickly and spread to other parts of the body. Checking the amount of HER2/neu on some types of cancer cells may help plan of treatment. Amplification and/or overexpression of HER-2/neu in human tumor tissue has been found in breast, ovarian, endometrial, colon, gastric or gastroesophageal junction, urothelial, bladder, salivary duct and cervix cancer. The degree of overexpression correlates with tumor progression, resistance to chemotherapy and a poor prognosis. Testing for this overexpression/amplification in tumor and its recurrence is very important. Immunohistochemistry and FISH are two important modern techniques which can identify overexpression of this protein in formalin fixed paraffin embedded tissue.

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Keywords: Her-2/neu overexpression, Immunohistochemistry of Her-2/neu, FISH, parrafin embedded tissue

Introduction

EGFR was the first discovered epidermal growth factor receptor.[1] *HER2* is so named because it has a similar structure to human epidermal growth factor receptor, or HER1. *Neu* is so named because it was derived from a rodent glioblastoma cell line, a type of neural tumor. *ErbB-2* was named for its similarity to *ErbB* (avian erythroblastosis oncogene B), the oncogene later found to code for EGFR.[2] *ERBB2,* a known proto-oncogene, is located at the long arm of human chromosome 17 (17q12).[2]

Growth factor binding results in receptor dimerization, subsequent tyrosine kinase activity and autophosphorylation of specific tyrosine residues. After that event downstream activation of signal transduction cascades occur and MAPK, Akt and JNK pathways become activated. It leads to DNA synthesis, cell proliferation, and differentiation. EGFR, also known as ErbB-1, and the three related receptors of the ErbB family: ErbB-2, ErbB-3, and ErbB-4. ErbB-2 is also known as HER2 in humans and neu in rodents. The HER-2/neu oncogene was first identified as a dominant transforming gene in chemically induced adrenal neuroblastomas of neonatal mice and was referred to as neu.[3,4] Subsequently, three groups independently identified the human homologue of this gene.[5,6] Sequence analysis of the gene demonstrated a close relation ship to the human epidermal growth factor receptor (HER-i) or *c-erbB oncogene.*[5,6] *Because of the similarities with HER-i, this* gene was considered to code for a membrane receptor.[5,6]

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Pathophysiology

Normal cells express 40,000 to 100,000 EGFR receptors, cancer cells may express up to 2,000,000 receptors.[1] Stimulation of overexpressed EGFR receptors may cause cancer by inducing cancer-cell proliferation while simultaneously blocking apoptosis. Cancer cells cause invasion and metastasis by activating invasion and metastasis of hyperproliferative cells and by stimulating tumor-induced neovascularization.[1]

Amplification and/or overexpression of HER-2/neu in human tumor tissue has been associated with a poor prognosis in cancers of breast,[7] ovary,[8] endometrium,[9] colon,[10] gastric or gastroesophageal junction,[11] urothelial,[12] bladder,[12] salivary duct[13] and cervix.[14] Amplification, also known as the over-expression of the *ERBB2* gene, occurs in approximately 15-30% of breast cancers, 7-34% of patients with gastric cancer and in 30% of salivary duct carcinomas.[2] The degree of overexpression correlates with tumor progression, resistance to chemotherapy and a poor prognosis.[1]

However, regarding involvement of HER2 protein 3+ expression in nonepithelial malignancies, it was very rare, often non-existent, in malignancies of non-epithelial origin. Out of 965 malignant melanoma cases, only one showed HER2 3+ expression. In 1,211 sarcomas of soft tissues and 1,136 neuroendocrine tumors, none exhibited 3+ HER2 protein expression. No HER2 3+ expression was detected in gastrointestinal stromal tumors (GIST), small cell lung cancers (SCLC), kidney cancers, and glioblastomas.[15,16]

Tests for Her-2

Immunohistochemistry and in situ hybridization (ISH, FISH) are the recommended methods for determining Her2 status for treatment with Her-2-targeted therapy. Neither method is 100% sensitive or specific.Updated ASCO-CAP (2013) guidelines have resulted in increased proportion of patients being eligible for Her2-targeted therapy. Her2-positive cases are not a homogeneous group – borderline positive cases may not be as responsive to Her-2-targeted therapy. Challenges in Her-2 laboratory testing include polysomy / co-amplification, and genetic heterogeneity.[17,18]

Immunohistochemistry (IHC) and HER-2 *in situ* hybridization are the most commonly used techniques for Her-2 expression. IHC can be done on formalin-fixed, paraffin-embedded tissue . Tests are usually performed on biopsy samples obtained by either fine-needle aspiration, core needle biopsy, vacuum-assisted breast biopsy, or surgical excision. Immunohistochemistry is used to measure the amount of HER2 protein present in the sample. The sample is given a score based on the cell membrane staining pattern. Specimens with equivocal IHC results should then be validated using fluorescence in situ hybridisation(FISH).[2] HER-2 scoring was reported per American Society of Clinical Oncology/College of American Pathologists (ASCO/CAP) guidelines published in 2007 and updated in 2013. IHC test was considered positive (IHC+) when IHC3+ was obtained above the guidelines defined thresholds; an IHC test was considered negative (IHC-) when IHC 2+ (equivocal), IHC 1+, or IHC 0 was obtained.[15,17,18,19]

HER2 *in situ* hybridization technique

In a study 37,992 samples were analyzed by IHC and 21,642 samples were also examined with ISH. FISH was used for evaluation of the *HER2* amplification status. FISH was performed with a probe specific for *HER2* (17q11.2-q12 region) and a probe for the pericentromeric region of chromosome 17. Interphase nuclei were examined and the

ratio of HER2 signals to chromosome 17 centromere signals were evaluated to indicate amplification status of this gene.[15]. *HER2/CEP17* ratio higher than 2.2 was considered amplified (ISH+), and HER2/CEP17 ratio between 1.8 and 2.2 (equivocal) in FISH or HER2/CEP17 ratio <1.8 in FISH was considered non-amplified (ISH-). [15,17]

HER2 amplification was also evaluated by CISH. The HER2 and chromosome 17 probes are detected using two color ISH in formalin-fixed, paraffin-embedded human cancer tissue specimens following staining on automated slide stainer, and visualized by light microscopy. Consistent with the CISH package insert, *HER2/CEP17* ratio higher than 2.0 was considered amplified (ISH+); HER2/CEP17 ratio <2.0 in CISH was considered non-amplified (ISH-). 11,670 patients had IHC and FISH; 9972 patient, IHC and CISH. IHC test was considered positive (IHC+) when IHC 3+ was obtained. ISH test was considered positive (ISH+) when the HER2/CEP17 ratio was >2.2 (by FISH) or 2.0 (by CISH).[15, 17,18,19]

The extracellular domain of HER2 can be shed from the surface of tumour cells and enter the circulation. Measurement of serum HER2 by enzyme-linked immunosorbent assay (ELISA) offers a far less invasive method of determining HER2 status than a biopsy and consequently has been extensively investigated. Results so far have suggested that changes in serum HER2 concentrations may be useful in predicting response to trastuzumab therapy.[2]

Clinical Significance

The ErbB family consists of four plasma membrane-bound receptor tyrosine kinases. One of which is erbB-2, and the other members being epidermal growth factor receptor, erbB-3 (neuregulin-binding; lacks kinase domain), and erbB-4. All four contain an extracellular ligand binding domain, a transmembrane domain, and an intracellular domain that can interact with a multitude of signaling molecules and exhibit both ligand-dependent and ligand-independent activity. Notably, no ligands for HER2 have yet been identified.[2]

HER2 has been firmly established in preclinical and clinical settings. Among all four HER family proteins, HER2 has the strongest catalytic kinase activity and functions as the most active signaling complex of the HER family after dimerization with other HER family members. [20,21] Overexpression of HER2 in breast cancer leads to increased homodimerization (HER2:HER2) and heterodimerization (e.g., HER2:HER3), which initiates a strong pro-tumorigenic signaling cascade.[22] Overexpression of HER2 protein drives malignant transformation in cell culture and transgenic mouse models.[23,24] The anti-HER2 antibody trastuzumab represents an effective, targeted therapy with significant efficacy in treatment of HER2-positive breast and gastric cancer.[25,26]

Regarding the mechanism of activation of EGFR, It starts by ligand binding at the extracellular domain which results in homo and heterodimerization, leading to phosphorylation, activation of downstream signaling pathways which upregulate expression of genes, proliferation and angiogenesis. Abnormalities in the expression of EGFR play an essential role in the development of different types of cancer. HER2 is the preferred heterodimerization partner for EGFR.; this biological characteristic together with structural homology has played a key role in the development of dual synthetic inhibitors against EGFR/HER2. [27]

Overactivation of the ErbB protein family, which is comprised of 4 receptor tyrosine kinase members, can drive the development and progression of a wide variety of malignancies, including colorectal, head and neck, and certain non-small cell lung cancers (NSCLCs). As a result, agents that target a specific member of the ErbB family have been developed for the treatment of cancer.[28]

Her2 targeted therapy include Herceptin (trastuzumab) and Others: pertuzumab (Perjeta), T-DM1 (Kadcyla), and lapatinib (Tykerb). Recent data shows that a combination of pertuzumab, trastuzumab, and docetaxel (PTD) improved progression free survival compared to patients who had only trastuzumab and docetaxel (TD).[29,30]

Recent development of HER2 mutation

Apart from gene amplification ,somatic HER2 (encoded by *ERBB2*) mutations, , have been reported recurrently in the literature. Mutations in HER2 are clustered in the extracellular, transmembrane and kinase domains. HER2 mutations have been found in non-small-cell lung cancers (NSCLC) and can direct treatment[31]. Also, HER-2 mutations are infrequent in a wide variety of cancers but targetable. As for example In breast cancers, activating mutations were identified as follows: G309A, D769H/Y, V777L, P780ins, V842I, and R896C,[32] L755S was associated with lapatinib resistance. All of these mutations were sensitive to the irreversible kinase inhibitor, neratinib. Recently, phase II SUMMIT trial, which is a HER2 mutant basket trial, showed mutation status can contribute to response to neratinib regardless of tumor type [33].

Conclusion

Checking the amount of HER2/neu on some types of cancer cells may help plan treatment. It's overexpression is mostly confined in malignant tumors of epithelial origin. Immunohistochemistry and FISH are two preferred techniques for identification of its overexpression/amplification. Targetting of Her-2/neu gene with proper drug is important for both treatment and recurrence of many cancer.

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