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Research Article

EVALUATION OF HER2/NEU OVER EXPRESSION IN BREAST CANCER

KANAGATHARA N*, KAVITHA K

Department of Pharmaceutical Technology, Bharathidasan Institute of Technology, Anna University, Tiruchirappalli.
Email: thara.biotech@gmail.com

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ABSTRACT

Genetic, molecular and poor prognostic conditions are some of the reasons behind high breast cancer incidences throughout the globe as well as in India, making it to be second highest recorded cancer among all cancer patients. This study tries to bring a correlation among the protein/receptor expression by Immunohistochemistry and corresponding gene status by Fluorescent In-Situ Hybridizations (FISH). The diagnosis of breast cancer is characterized by the over expression of Her-2 receptors on the metastased cells of breast. Herceptin (trastazumab) being a vital drug used, inhibits the tyrosine kinase activity by preventing dimerisation of the Her-2 receptors. The treatment modules depend on the amount of Her-2 expression in the tumor tissue. Hence, Herceptin Monoclonal antibody is suggested for breast cancer patients, as a targeted therapy in initial stage 1+ itself to stop further cell division in breast cancer and also to inhibit metastasis stage.

Keywords: IHC, FISH, Herceptin, Targeted therapy, Her2/neu.

INTRODUCTION

Cancer is a kind of disease in which a group of cells undergo uncontrollable cell growth, invasion or metastasis. Worldwide, breast cancer is the second most common type cancer after lung cancer. In 2004 breast cancer cost 519000 deaths worldwide [1]. The term breast cancer refers to a malignant tumor that has developed from cells in the breast. Usually breast cancer either begins in the cells of the lobules, which are the milk producing glands or the ducts, the passage, the drain milk from the lobules to the nipple. If the cancer cells get into lymph nodes, they then have a pathway into other parts of the body.

The breast cancers stage refers to the development of cancer cells that spread beyond the original tumor. Breast cancer is always caused by a genetic abnormality, only 5 to 10 % of cancer is due to an abnormality inherited from father or mother, about 90 percent of breast cancer are due to genetic abnormalities that happen as a result of aging process and wear and tear of life in general. Her-2 is a gene that sends control signals to cells, telling them to grow, divide and make repairs. A healthy breast cell has 2 copies of the Her-2 gene. Some kinds of breast cancer get started when a breast cell has more than 2 copies of the gene and those copies start over-producing the HER-2 protein. Her-2/neu (also known as ErbB-2, ERBB2)[2] stands for "Human Epidermal growth factor Receptor 2" and is a protein family, more commonly known as the epidermal growth factor receptor family. Her-2/neu has also been designated as CD340 (Cluster of differentiation 340) and p185. In some cancers, notably some breast cancers, the Her-2 receptor is defective and struck in the "on" position and causes breast cells to reproduce uncontrollably, causing breast cancer [3].The Her-2 (also known as Her-2/neu and ErbB2 gene) is amplified in 20-30% of early-stage breast cancers, which makes it overactive (or over expressed). Her-2 passes through the cell membrane and sends signals from outside the cell to the inside. Signalling compounds called mitogens arrive at the cell membrane and bind to the outside part of Her-2. Her-2 is activated and sends a signal to the inside of the cell. The signal passes through different biochemical pathways. This includes the P13KAkt pathway and the MAPK pathway.

Trastuzumab (Herceptin) [4] is a monoclonal antibody that interferes with the Her-2/neu receptor. When it binds to defective HER-2 proteins, the HER-2 protein no longer causes the breast cells to reproduce uncontrollably. This increases the survival of people with cancer. Herceptin (trastuzumab) is not chemotherapy or hormonal therapy. Herceptin is a type of targeted cancer therapy known as a monoclonal antibody. Trastuzumab is a humanized monoclonal antibody which binds to the domain IV of the extracellular segment of the Her-2/neu receptor. Cells treated with trastuzumab undergo arrest during the G1 phase of the cell cycle so there is reduced proliferation. Automated Tissue Image Systems by laboratories for mechanized and automated processing of specimen thereby reducing process variability avoiding equivocal cases and enhancing probability of Trastuzumab therapy.

MATERIALS AND METHODS

The Biopsy specimen[5] was obtained from the patient of Breast cancer and the specimen was observed and the tumour part was cut out and used for further analysis.10% formalin was used to fix the tissue sample. 14 samples were taken randomly and the techniques IHC and FISH were done simultaneously by the following methodology and the results were reported on the basis of protein expression for IHC and on ratio basis for FISH. The FFPE [6] blocks were prepared by placing the tissue samples in the moulds along with the liquid embedding material which was then hardened. The tissue sections were cut with a microtome for IHC and FISH studies.

IHC (immunohistochemistry) Staining

The 6 micron section [7] was taken on Poly-L-Lysine (PLL) coated slides with one positive control for each marker. The Slides were incubated at 60°C overnight. The Slides were treated with xylene for deparafinaiztion of slides and then the slides were treated with alcohol for de hydration. The slides were then rinsed with distilled water for 5 min. A 20 minute treatment with primary blocking agent (3% hydrogen peroxide) was given. For antigen retrieval, the slides were treated in a water bath for 1 hour. The slides were then cooled down to room temperature. The slides were washed in TBST (tris buffered saline in Tween 20). A 1 hour treatment with secondary blocking agent (3%Bovine Serum Albumin) was given. The slides were treated with primary antibody (polyclonal rabbit human C-erbB-2 Oncoproteins) for 1 hour. The slides were again washed in TBST. The slides were treated with secondary antibody (monoclonal rabbit secondary antibody) for 30 minutes. The slides were washed with TBST. The slides were then treated with DAB (3, 3'-diammino benzedine) for 4 minutes. The slides were stained with haematoxylin for 20 seconds. They were then treated with 96% alcohol for dehydration. The slides were treated with xylene for clearing. DPX was added to mount the slide and cover slip was placed. The slides were observed in microscope to determine the Her2/neu protein level.

FISH (fluorescent in-situ hybridisation)

The FFPE [8] sections were taken in PLL coated slides and incubated at 60°c overnight. The slide was then kept in xylene for de paraffinisation. The slide is then kept in alcohol for dehydration. Then, the thermobrite moisten was set, to that 2xSSC was added to 75°c thermobrite and kept for 10 minutes. Then proteinase k was treated in thermobrite at 45°c for 10 minutes. After that the

thermobrite was washed with 2xSSC for 2 minutes. Then dehydration of the slide is carried out with alcohol for 2 minutes. Fluorescent lamp has been on and then DAPI (1-6'-diammino-4-phenol indole) was added on it to check the digestion. Then to that 2x SSC was added for 3 minutes. HER2 vices probe was thawed. After that 10µl of probe was added, and then covered with a cover slip and sealed with a rubber cement. Then the slide is kept for denaturation at 80°c for 10 minutes. After that the slide is kept for hybridisation overnight. Then the slide is kept in the solution of 2x SSC for 2 minutes for washing. Again, dehydration was done in alcohol for 2 minutes. 2µl of DAPI was added and covered with a cover slip and stored at 4°c till counting was done. Then, the slide was observed under fluorescent microscope to determine the Her2 gene amplification status.

RESULTS

Her2/neu gene overproduction causes breast cancer. There are different stages of breast cancer in Fig 1 the breast cancer level is 1+ which signifies that the tumour is 2cm or les than that,

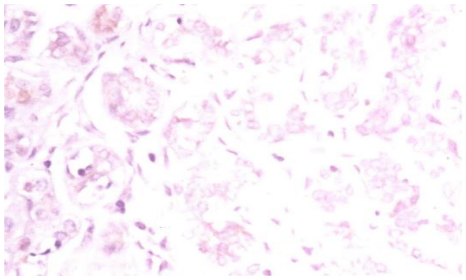


Fig. 1: Histopathological study of breast cancer 1+ level cells

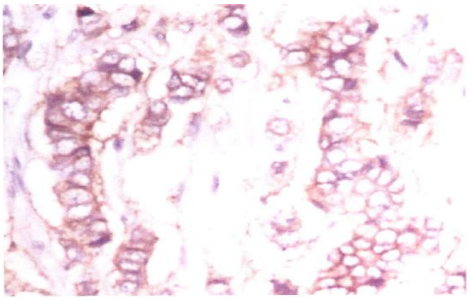


Fig. 2: Histopathological study of breast cancer 2+ level cells

whereas in Fig 2 the cancer level is 2+ where the cell size is large and the lymph nodes, chest cell wall and skin of the breast has been affected. Fig 3 represents the cancer level as 3+ where the cancer level has spread or metastasized to other parts of the body.

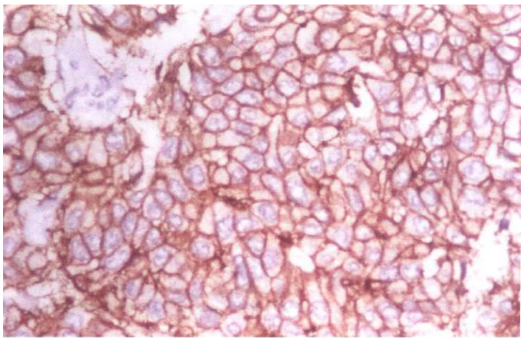
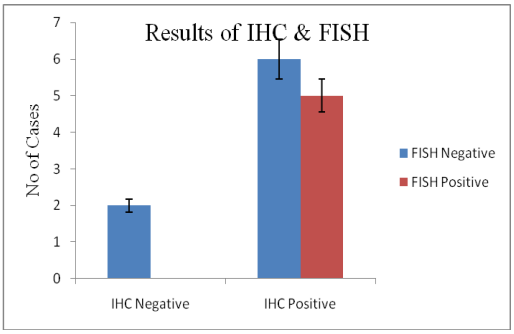


Fig. 3: Histopathological study of breast cancer 3+ level cells

DISCUSSION

The IHC Negative samples are 2 where the protein level is only low corresponding with the low level gene amplification of Her-2 whereas in IHC Positive samples 11[9], high protein level as well as gene amplification was observed(table 1).

Table 1: Gene amplification level



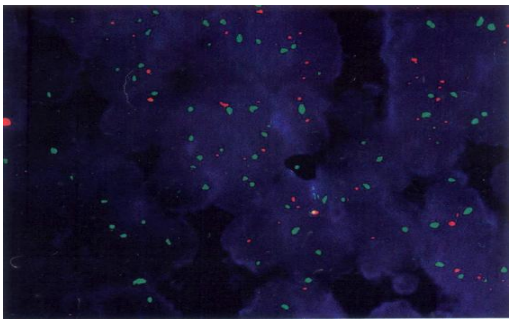


Fig. 4: Polysomy of chromosome 17

In the samples where the protein level was observed to be high, had low gene amplification [10, 11] in FISH, this signifies the presence of polysomy (Fig 4) status of the chromosome 17 (Fig 5)

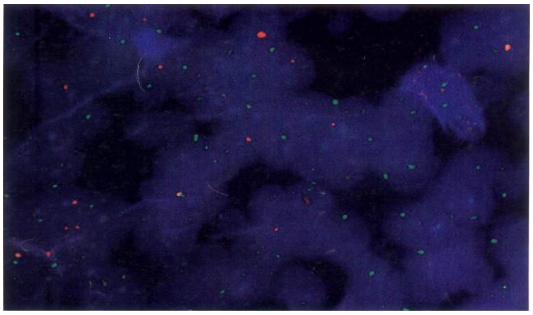


Fig. 5: Amplification level of chromosome 17

Downs syndrome is an example of polysomy where affected individuals possess three copies of chromosome 21 [12]. In polysomy condition, the higher chromosomal signal thus reduces the ratio value (table 1). Her2 gene and chromosome 17 copy were determined by dual colour FISH (Fig 6).

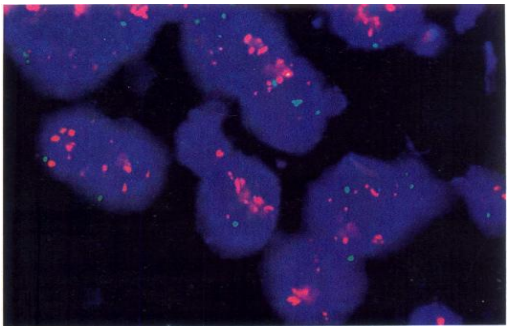


Fig. 6: Copy number of the chromosome17

Copy number [13] variation is a segment of Deoxyribo Nucleic Acid in which copy number differences have been found by comparison of two or more genomes [14]. Factors such as size, shape, orientation, % similarity and the distance between the copies render them susceptible. Copy number variation can be discovered by cytogenetic

techniques such as FISH, Comparative Genomic Hybridisation, and Array Comparative Genomic Hybridisation by virtual karyo typing with Single Nucleotide Polymorphism arrays. It is estimated that approximately 0.4% of the genomes of unrelated people typically differ with respect to copy number [15].

CONCLUSION

The Her-2/neu gene over expression in breast cancer is evaluated and the her2/neu gene protein level is determined by IHC and the gene amplification status is determined using FISH. Herceptin Monoclonal antibody is suggested for breast cancer patients, as a targeted therapy in initial stage 1+ itself to stop further cell division in breast cancer and also to inhibit metastasis stage.

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