

ORIGINAL ARTICLE

HER-2/neu expression in germ cell tumours

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14 May 2002**Aims:** To determine the rate of HER-2/neu positivity of germ cell tumours by immunohistochemistry (IHC) and by fluorescence in situ hybridisation (FISH).**Patients/Methods:** Ninety six archival, paraffin wax embedded pathology specimens were chosen from four groups of germ cell tumours. IHC for HER-2/neu was performed with the HercepTest kit; FISH analysis was performed with the INFORM assay and confirmed with a centromere 17 probe.**Results:** Twenty two of 96 specimens overexpressed the HER-2/neu protein when measured by IHC. Only three specimens showed HER-2/neu gene amplification by FISH. There was no correlation between the results obtained by IHC and FISH.**Conclusions:** The lack of concordance between IHC and FISH makes it unlikely that overexpression of the HER-2/neu protein in germ cell tumours is of prognostic or therapeutic relevance. Because of the low rate of HER-2/neu gene amplification in germ cell tumours, a clinical trial of trastuzumab treatment in patients with germ cell tumours is not warranted.

Germ cell tumours are the most chemosensitive of all solid tumours and are highly curable with cisplatin based chemotherapy.¹ However, although 95% of patients with germ cell tumours overall are cured with current treatment protocols, approximately 30% of patients with disseminated disease fail to achieve complete remission or subsequently relapse, requiring further salvage treatment. Despite the improvement in salvage treatment with the advent of high dose chemotherapy with stem cell rescue, and the growing recognition of the role of surgery, over 50% of those patients who relapse ultimately die of their disease. Further improvements in the cure rate will require increased understanding of the biology of germ cell tumours.

Recent scientific attention has focused on the role of growth factors in the progression of cancer. HER-2/neu is an epidermal growth factor receptor that is overexpressed on the cell surface of approximately 25–30% of breast cancers.² This expression correlates with a relatively poor prognosis for patients with breast cancer; it is associated with a shorter disease free survival and overall survival.³

“Over 50% of those patients who relapse ultimately die of their disease”

The increased expression of HER-2/neu in breast cancer is most often the result of HER-2/neu oncogene amplification; protein overexpression in the absence of gene amplification is rare. Specific antibodies to the extracellular domain of the protein encoded by the HER-2/neu gene inhibit the growth of some HER-2 overamplified tumours. As such, HER-2/neu has proved to be a useful therapeutic target in breast cancer; treatment of patients with HER-2/neu amplified tumours with the monoclonal antibody trastuzumab results in a 19–34% clinical response rate.⁴

Overexpression of HER-2/neu has been reported in many other epithelial malignancies, including cancers of the lung,⁵ prostate,^{6,7} bladder,^{8,9} pancreas,^{10,11} and oesophagus,¹² and sarcoma.^{13,14} However, there is no evidence so far that HER-2/neu expression is of prognostic relevance in these malignancies. There is also no evidence of a correlation between immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) results in any tumours except breast.

Although currently there is no evidence that trastuzumab is of therapeutic benefit in other malignancies, clinical trials of treatment with trastuzumab in many tumours are exploring this possibility.

The potential role of HER-2/neu in germ cell tumours is unknown. A pilot study analysed biopsy or surgical specimens from 10 patients with germ cell tumours whose tumours progressed after initial chemotherapy. These tumours were tested immunohistochemically with monoclonal anti-HER-2/neu antibody and scored from 0 to 3+. Two of these tumours were scored as 2+, one tumour was 1+, and the other seven tumours were negative (0).¹⁵

The objectives of our study were to determine the incidence of HER-2/neu protein overexpression and gene amplification in both newly diagnosed and refractory germ cell tumours. These results would then be compared to determine the correlation between IHC and FISH in germ cell tumours. We also assessed the feasibility of Her-2/neu directed treatment in germ cell tumours.

MATERIAL AND METHODS

Ninety six paraffin wax embedded germ cell tumours were selected from the pathology archive. Samples were chosen from four groups to represent the spectrum of clinical behaviour of germ cell tumours, namely: (1) primary orchiectomy specimens (22 patients), (2) post chemotherapy retroperitoneal lymph node resections with germ cell carcinoma in the resected specimen (PCRPLND; 25 patients), (3) late relapse (≥ 2 years after diagnosis; 29 patients), and (4) primary mediastinal germ cell tumours (20 patients). Sections (4 μ m thick) were cut from archived tumour blocks and mounted on positively charged slides for analysis. All samples were analysed both by IHC for HER-2/neu protein overexpression and by FISH for HER-2/neu gene amplification in concordance with published testing guidelines.¹⁶ Approval was obtained from the institutional review board at Indiana University to access tissue samples and clinical records from these patients.

Abbreviations: FISH, fluorescence in situ hybridisation; IHC, immunohistochemistry; PCRPLND, post chemotherapy retroperitoneal lymph node resections with germ cell carcinoma in the resected specimen

Table 1 Immunohistochemistry results

| | Orchiectomy (n=22) | PCRPLND (n=25) | Late relapse (n=29) | Primary mediastinal (n=20) |
|-------|-----------------------|-------------------|------------------------|----------------------------------|
| 2+ | 0 | 5 | 4 | 5 |
| 3+ | 0 | 3 | 3 | 2 |
| Total | 0 (0%) | 8 (32.0%) | 7 (24.1%) | 7 (35.0%) |

PCRPLND, post chemotherapy retroperitoneal lymph node resections with germ cell carcinoma in the resected specimen.

IHC was performed with the HercepTest kit (Dako, Carpinteria, California, USA), according to the manufacturer's instructions. A pathologist who was blinded to tumour type and FISH results evaluated all IHC specimens. The staining intensity was scored from 0 to 3+ using the breast cancer HER-2/neu scoring system, with 2+ or 3+ staining considered positive for protein overexpression. Samples with mixed histology were considered positive if any portion of the tumour exhibited HER-2/neu staining, including those tumours in which only teratoma stained for HER-2.

HER-2/neu gene amplification was assessed by FISH using the INFORM HER-2/neu assay (Ventana, Tuscon, Arizona, USA), according to the procedure specified by the manufacturer. The INFORM HER-2/neu kit is Food and Drug Administration approved for FISH analysis of HER-2/neu gene copy number. A pathologist marked each slide for histological areas representing tumour. All slides were baked at 65°C for four hours, dewaxed in xylene, and dehydrated in 100% ethanol. Pretreatment consisted of protease (25 mg/ml) digestion. Cells and the biotin labelled HER-2/neu probe were co-denatured at 75°C for 15 minutes and hybridised overnight at 37°C. Detection was accomplished with fluorescein labelled avidin and a DAPI/antifade counterstain. Non-overlapping nuclei within the target area were analysed using a DMR fluorescent microscope (Leitz). A total of 40 nuclei (20 each from two different observers who were blinded to IHC results) were scored for signals. The mean numbers of signals recorded by each observer were combined and divided by two for a combined average. An average of < 4 signals/nucleus represented an absence of HER-2/neu gene amplification; a combined average of ≥ 4 signals/nucleus suggested gene amplification.

Positive results based on the INFORM HER-2/neu probe (≥ 4 signals/nucleus) were confirmed using a centromere 17 probe (CEP 17; Vysis, Downer's Grove, Illinois, USA). The procedure followed the manufacturer's instructions, except that treatment with 0.3% formaldehyde was omitted. In total, 60 uniform nuclei (30 for each observer) from the previously analysed target area were scored. Each observer calculated a ratio of HER-2/neu signals to control (centromere) signals. These values were combined and divided by two. A mean ratio of < 2 HER-2/neu signals to control signals represented the absence of HER-2/neu gene amplification. A mean of ≥ 2 indicated gene amplification. In addition, several specimens were analysed using the PathVysion HER-2/neu probe kit from Vysis. This kit includes the HER-2/neu probe labelled with spectrum orange and the CEP 17 centromere probe

labelled with spectrum green. The assessment of amplification was also based on a ratio of HER-2/neu signals to centromere signals. A signal ratio of < 2 was considered non-amplified and a signal ratio of > 2 was considered amplified.

RESULTS

Table 1 shows the IHC results. None of the primary orchiectomy specimens exhibited HER-2/neu overexpression. However, seven of the 20 patients with primary mediastinal tumours had protein overexpression. The rate of HER-2/neu overexpression was similar in patients with post chemotherapy germ cell carcinoma in the PCRPLND specimen or recurrent (late relapse) disease.

In contrast, HER-2/neu gene amplification was identified in only four tumours (table 2). Three of these samples were from the late relapse group, the other was a primary mediastinal germ cell tumour. Of the three late relapse samples, two had INFORM HER-2/neu to centromere 17 probe ratios of 2.75 and 2.08 signals/nucleus, and the other had a PathVysion ratio of 2.05 signals/nucleus. These samples had IHC expressions of 3+, 1+ and 3+, respectively. The FISH positive primary mediastinal germ cell tumour sample had an INFORM average of 4.4 nuclei/cell. However, the ratio of HER-2/neu signals to centromere 17 in this tumour was 1.93; IHC expression was 0. This specimen was reclassified as non-amplified.

DISCUSSION

Although HER-2/neu was overexpressed in 22 of the 96 patients with germ cell tumours, amplification of the HER-2/neu gene in these patients was rare (three of 96). Given the lack of concordance between protein expression and gene amplification, it is unlikely that overexpression of the HER-2/neu protein in germ cell tumours is of prognostic or therapeutic relevance.

"Because immunohistochemistry (IHC) positivity does not correlate with the amplification of HER-2/neu in germ cell tumours, patients who are IHC positive but fluorescence in situ hybridisation negative are unlikely to derive clinical benefit from treatment with trastuzumab"

There are several possible explanations for the lack of concordance between IHC and FISH in germ cell tumours. An analysis of 85 invasive breast cancer samples tested for HER-2/neu by IHC and FISH showed that the false positive IHC rate in breast tumours is 11% when IHC is 3+ and 76% when IHC is 2+.¹⁷ Importantly, patients with HER-2/neu amplification by FISH are more likely to respond to trastuzumab (relative risk, 19–34%) than are patients without HER-2/neu amplification (relative risk, 0–7%).⁴ The IHC procedure and antigen retrieval methods used in our study were developed for the analysis of breast cancer specimens. Adopting these techniques for use in germ cell tumours may have resulted in a higher false positive rate than is seen in breast cancer.

Another proposed hypothesis for the discrepancy between IHC and FISH results is that post translational modifications may lead to overexpression of the HER-2/neu protein without

Table 2 Immunohistochemistry (IHC) and fluorescence in situ hybridisation (FISH) results

| | Orchiectomy | PCRPLND | Late relapse | Primary mediastinal | Total |
|---------------|-------------|---------|--------------|------------------------|-------|
| IHC positive | 0/22 | 8/25 | 7/29 | 7/20 | 22/96 |
| FISH positive | 0/22 | 0/25 | 3/29 | 1/20 | 4/96 |

PCRPLND, post chemotherapy retroperitoneal lymph node resections with germ cell carcinoma in the resected specimen.

Take home messages

- Using immunohistochemistry (IHC), 22 of 96 germ cell tumours overexpressed HER-2/neu protein, whereas only three of 96 specimens showed HER-2/neu gene amplification by fluorescence in situ hybridisation (FISH)
- This lack of a correlation between IHC and FISH makes it unlikely that overexpression of the HER-2/neu protein in germ cell tumours is of prognostic or therapeutic relevance
- Because of the low rate of HER-2/neu gene amplification in germ cell tumours, a clinical trial of trastuzumab treatment in patients with germ cell tumours is not warranted

HER-2/neu gene amplification. However, recent data showing a correlation between mRNA expression and HER-2 gene copy number by FISH suggest that lack of concordance is the result of false positive IHC results rather than transcriptional regulation.¹⁸

Because IHC positivity does not correlate with the amplification of HER-2/neu in germ cell tumours, patients who are IHC positive but FISH negative are unlikely to derive clinical benefit from treatment with trastuzumab. Based on the rate of gene amplification in germ cell tumours, very few chemotherapy refractory patients in the USA each year would be eligible for trastuzumab treatment. Therefore, a clinical trial of trastuzumab in patients with germ cell tumours is not feasible.

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