Her-2/neu oncogene amplification in clinically localised prostate cancer

J D Oxley, M H Winkler, D A Gillatt, D S Peat

Aim: To examine the incidence of Her-2/neu oncogene amplification in clinically localised prostate cancer using in situ hybridisation.

Methods: One hundred and seventeen patients, who had undergone radical prostatectomy, were identified and in situ hybridisation was performed on formalin fixed, paraffin wax embedded tissue using the Quantum Appligene probe for Her-2/neu. The enzyme peroxidase was used to detect the probe because this enabled a permanent record to be kept. Tumours in which there were five or more signals in each nucleus in > 20% of the tumour cells were considered to have a significantly increased copy number. A serial section from these tumours was then hybridised with the chromosome 17α satellite probe. The ratio of the percentage of cells showing an increase in Her-2/neu copy number to the number showing polysomy for chromosome 17 was calculated. A ratio above 2 was considered amplified.

Results: Biochemical recurrence occurred in 50 (43%) patients and 24 (21%) had clinical recurrence. In situ hybridisation for Her-2/neu was accessible in 114 (97%) patients. A significant increase in copy number was present in two patients (1.75 %), but chromosome 17 hybridisation showed that the increase was the result of polysomy rather than true amplification. Both these patients had a Gleason score of 7 and stage T3; they also had recurrent clinical disease with distal metastasis within two and 19 months.

Conclusions: Increased Her-2/neu oncogene copy number appears to be rare in clinically localised prostatic adenocarcinoma and is related to chromosome 17 polysomy rather than true amplification. As a result, it would not be a useful biomarker for identifying those patients who will have recurrences after radical prostatectomy.

The advent of a humanised monoclonal antibody to the Her-2/neu protein, Herceptin (trastuzumab), and its use as an adjuvant therapeutic agent has stimulated even greater interest in the Her-2/neu oncogene. Recent studies in human prostate xenograft models showed a response to Herceptin in androgen dependent tumours, but not in androgen independent tumours.

The aim of our study was to examine the amplification of Her-2/neu in the largest series to date, using a modification of the FISH method in which enzymatic techniques rather than fluorescent detection are used.

Materials and Methods

Patients

One hundred and seventeen men with clinically localised biopsy confirmed adenocarcinoma of the prostate were included. The median age of the patients was 65 years (range, 50–73). The patients underwent either radical retropubic or perineal prostatectomy at our institution or at the Western General Hospital, Weston super Mare, between October 1987 and December 1998. On digital rectal and clinical examination all were considered to have organ confined disease and all had a negative preoperative isotope bone scan. Ten (9%) patients received preoperative hormonal neoadjuvant treatment. Histopathological evaluation was performed using the Gleason system.

Abbreviations: FISH, fluorescent in situ hybridisation; PSA, prostate specific antigen, SSC, sodium chloride/sodium citrate solution
In situ hybridisation

JO and DP identified formalin fixed, paraffin wax embedded tissue blocks containing representative areas of tumour with the highest Gleason grade. Sections (5 µm thick) were mounted on to sialinised slides. The sections were dewaxed in xylene, washed twice in 100% ethanol, and air dried. They were then placed in the pretreatment solution of 30% sodium bisulphite (Sigma Aldrich, Poole, Dorset, UK) for 45 minutes at 45°C. Next, the slides were washed in 2× sodium chloride/sodium citrate (SSC; 0.3M sodium chloride, 0.03M sodium citrate at pH 7.0). Protein digestion was then carried out by immersing the slides in proteinase K (Sigma Aldrich) at 100 µg/litre for between 30 and 50 minutes at 45°C. The digestion time was calculated based on the degree of propidium iodide uptake, according to the manufacturer’s instructions (propidium iodide antidote; Quantum Appligene Lifescreen, Harefield, Middlesex, UK). After digestion, the slides were dehydrated in 70%, 90%, and 100% ethanol and then air dried. Next, 8 µl of Her-2/neu digoxigenin labelled probe/hybridisation mix was added to the tissue (Quantum Appligene Lifescreen). A 22 mm² coverslip was then applied and rubber solution used to seal the coverslip. The slides were placed on a hotplate at 70°C for five minutes to denature the probe and the tissue DNA. The slides were then incubated in a humidified chamber at 37°C overnight. The next day the rubber solution was peeled off and the slides were soaked in 2× SSC to remove the coverslip. This was followed by a stripping wash in 2× SSC (pH 7.0) at 73°C for five minutes. The slides were washed in Optimax wash buffer (1× SSC with 0.1% Tween 20) and then placed on a hotplate at 70°C for five minutes to denature the coverslip. The slides were then placed in the pretreatment solution of 30% sodium bisulphite (Sigma Aldrich, Poole, Dorset, UK) and then air dried.

In situ hybridisation was successful in 114 of the 117 patients and this was at a low level (23% and 38%) (fig 1B). The ratios to chromosome 17 were both less than 2 (0.8 and 1.6). These two patients both had a Gleason score of 7; one had a stage pT3A tumour, which clinically recurred with distal metastasis after 19 months. The other had a stage pT3B tumour, which clinically recurred after two months with distal metastasis.

## DISCUSSION

Prostatic adenocarcinoma appears to be increasing in men under the age of 60 in the UK, even when the effects of the increased detection of subclinical cases are accounted for. Although many biological markers have been studied retrospectively none is used routinely in a prospective manner. The most reliable predictor of recurrence is still the Gleason score and stage, although the expression of p53 and bcl-2 also appears to be useful. Developments in prostate cancer have lagged behind those made in breast cancer—for example, immunohistochemical assessment of oestrogen receptor status is now undertaken in every breast tumour in the UK. In the USA, the Food and Drug Administration (FDA) has approved the use of two FISH assays of Her-2/neu amplification in certain clinical situations in breast cancer. One of these FISH assays (INFORM™) uses the Quantum Appligene probe (used in our study) (Ventana Medical Systems, Tuscon, Arizona, USA), whereas the other assay (PathVision™) uses the Vysis probe (Vysis, Downers Grove, Illinois, USA). The availability of these probes and the therapeutic agent Herceptin has led to increased studies of Her-2/neu in all tumours.

There was a tendency for the signal to be located at the nucleolar membrane (fig 1A). A significant increase in the Her-2/neu gene copy number was seen in only two (1.75%) of the patients and this was at a low level (23% and 38%) (fig 1B). The ratios to chromosome 17 were both less than 2 (0.8 and 1.6). These two patients both had a Gleason score of 7; one had a stage pT3A tumour, which clinically recurred with distal metastasis after 19 months. The other had a stage pT3B tumour, which clinically recurred after two months with distal metastasis.

"The most reliable predictor of recurrence is still the Gleason score and stage, although the expression of p53 and bcl-2 also appear to be useful"

Early studies in prostatic carcinomas using immunohistochemistry to detect Her-2/neu gene products showed varied results. This probably resulted from the different antibodies...
used, variations in antigen retrieval, and small patient numbers. Similar variations have been found in breast cancers, but with the introduction of a standardised technique (HercepTest™) these problems should be resolved. To date, no study of prostate cancer using this technique has been published.

Using FISH, a high percentage of gene amplification has been found in those cases of breast cancer showing protein overexpression. In prostate cancer, one group has found amplification of the Her-2/neu oncogene in up to 44% of cases, and this was associated with advanced pathological stage and higher Gleason score. However, more recent work by that group showed a lower amplification rate of 10–25% (personal communications, JS Ross, 2000). Using the Vysis probe, Mark et al found an amplification rate of 9%. The Vysis Her-2/neu probe has a great advantage over the Quantum Appligene probe in that it also contains an internal control of a chromosome 17q satellite probe. As a result, two colour FISH can be used and this allows the ratio of chromosome 17 number to Her-2/neu copy number to be calculated. The major drawback of two colour FISH is its reliance on computer assisted analysis, which may restrict the application of this technology to larger centres.

The largest study of prostate cancer and Her-2/neu amplification used a combination of FISH using the Vysis probe and microarrays. This technique allowed 262 separate tumours to be assessed by taking small samples (0.6 mm in diameter) and mounting them on a single slide, which was then used for FISH. Microarrays have the great advantage of being able to screen large numbers of tumours for gene amplifications, but there can be sampling error, because most tumours are heterogeneous. Bubendorf et al tried to take this into account by using tumours from different stages of the disease—from localised to metastatic. They found Her-2/neu was not amplified at any stage of the disease.

The differences in amplification rates in these studies appear to result from the definition of amplified used by the different investigators (table 1). Ross et al did not control for polisomy, whereas Mark et al used a ratio of 1.5 and Bubendorf et al used a ratio of 3. Although no cases of amplification were found by Bubendorf et al there was a single case in the study by Mark et al with a ratio of 3.

Our study is the largest to date using complete sections of tumour tissue, and S Swann, C Abbott, and K Sibley for their technical work. The Southmead Research Foundation funded this project.

**ACKNOWLEDGEMENTS**

We thank Dr A Charles for his advice, Dr D Patterson for providing tumour tissue, and S Swann, C Abbott, and K Sibley for their technical work. The Southmead Research Foundation funded this project.

---

### Table 1

<table>
<thead>
<tr>
<th>First author</th>
<th>Patient numbers</th>
<th>Probe</th>
<th>Criteria for amplification</th>
<th>% Amplified</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ross</td>
<td>113</td>
<td>Quantum Appligene</td>
<td>≥5 signals in ≥20%</td>
<td>41%</td>
</tr>
<tr>
<td>Mark</td>
<td>86</td>
<td>Vysis</td>
<td>Ratio ≥1.5</td>
<td>9%</td>
</tr>
<tr>
<td>Bubendorf</td>
<td>262 tumour microarrays</td>
<td>Vysis</td>
<td>Ratio ≥3</td>
<td>0%</td>
</tr>
<tr>
<td>Oxley</td>
<td>114</td>
<td>Quantum Appligene</td>
<td>≥5 signals in ≥20%</td>
<td>1.75%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ratio ≥2</td>
<td>0%</td>
</tr>
</tbody>
</table>

---

### Take home messages

- Increased Her-2/neu oncogene copy number is rare in clinically localised prostate adenocarcinoma and is related to chromosome 17 polysomy rather than true amplification
- Her-2/neu copy number is not a useful biomarker for identifying those patients who will have recurrences after radical prostatectomy

---

### Authors’ affiliations

J D Oxley, D S Peat, Department of Cellular Pathology, Southmead Hospital, Weston-by-Trym, Bristol BS10 5NB, UK

M H Winkler, D A Gillatt, Department of Urology, Southmead Hospital

### REFERENCES


---

**www.jclinpath.com**
Her-2/neu oncogene amplification in clinically localised prostate cancer

J D Oxley, M H Winkler, D A Gillatt and D S Peat

J Clin Pathol 2002 55: 118-120
doi:

Updated information and services can be found at:
http://jcp.bmj.com/content/55/2/118

These include:

References
This article cites 12 articles, 4 of which you can access for free at:
http://jcp.bmj.com/content/55/2/118#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections

- Molecular genetics (355)
- Prostate cancer (71)
- Urological cancer (164)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/