Detection of topoisomerase IIα (TopoIIα) gene amplification and deletion by chromogenic in situ hybridization (CISH™) in archival breast carcinoma

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INTRODUCTION
The TopoIIα gene is located at chromosome 17q21.2, close to the HER2 gene, which is the most commonly amplified oncogene in breast cancer. TopoIIα gene amplification and deletion are frequently observed in HER2-amplified breast cancer and are associated with the most commonly amplified oncogene in breast cancer. TopoIIα protein is the target for trastuzumab (Herceptin®) therapy, and TopoIIα protein overexpression is an indicator for trastuzumab therapy. However, TopoIIα protein level failed to predict response to topoII inhibitor chemotherapies in metastatic breast cancer. Further study in breast cancer patients showed that amplification of TopoIIα gene in primary tumors is a strong predictor of clinical response to topoII inhibitor in metastatic disease⁶. Therefore, accuracy and ease for routinely used assay to evaluate TopoIIα gene status becomes important.

We have developed CISH using HER2 or TopoIIα DNA probe generated by Subtracted Probe Technology (SPT). With SPT technology, repetitive DNA sequences e.g. Alu and LINE elements, which may consist of up to more than 40% of template and cause unspecific hybridization, are quantitatively removed. Therefore, the final probe is very specific and the need for blocking of non-specific hybridization with Cot-1 DNA in traditional FISH probes is eliminated. The goal of this study is to determine if CISH using SPT TopoIIα DNA probe can be assessed for TopoIIα amplification as well as deletion in archival breast cancer tissues.

MATERIAL AND METHOD

Tumors: FFPE (Formalin-Fixed, Paraffin-Embedded) tissue sections from 29 of breast cancer cases with HER2 amplification and 30 of breast cancer cases without HER2 gene amplification were studied.

SPT TopoIIα DNA Probe (Zymed): 170 kb in size. Digoxigenin labeled. The distance between SPT HER2 probe to TopoIIα probe is about 580 kb.

CISH: CISH was done on 4mm tissue sections. Briefly, the sections were de-paraffinized, followed by heat pretreatment and enzyme digestion (CISH Tissue Pretreatment Kit, Zymed). Ready-to-use DIG-labeled HER2 or TopoIIα probe (Zymed) or biotin-labeled chromosome 17 centromeric probe (bio-chr. 17cen. Zymed) was applied on adjacent sections before the denaturation and hybridization. After the stringent wash, the HER2 or TopoIIα probe was detected with sequential incubation of FITC conjugated anti-digoxigenin antibody, HRP-conjugated anti-FITC antibody, and DAB (CISH Detection Kit, Zymed, figure 1) or with sequential incubation of mouse anti-DIG, polymerized HRP anti mouse (CISH Polymer Detection Kit, Zymed, figure 2). The chr.17cen probe was detected with HRP-streptavidin and DAB (CISH Centromere Detection Kit, Zymed, figure 3).

Table 1: Interpretation of HER2 and TopoIIα: CISH results

<table>
<thead>
<tr>
<th>HER2 gene status</th>
<th>HER2 CISH</th>
<th>TopoIIα gene status</th>
<th>TopoIIα CISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amplification</td>
<td>≥6 copies or clusters of amplicon per nucleus in &gt;50% of cancer cells.</td>
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<td>≥6 copies or clusters of amplicon per nucleus in &gt;50% of cancer cells.</td>
</tr>
<tr>
<td>No amplification</td>
<td>&lt;6 copies per nucleus in &gt;50% of cancer cells.</td>
<td>No amplification</td>
<td>TopoIIα copies &lt; chr.17cen probe copies.</td>
</tr>
<tr>
<td>(Diploid or aneuploid)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bio-chr.17cen probe was applied to CISH to confirm a low level gene amplification when 6-10 HER2 copies per nucleus in &gt;50% of cancer cells.</td>
<td>Diploid or aneuploid</td>
<td>TopoIIα copies &lt; chr.17cen probe copies and ≥6 copies per nucleus in &gt;50% of cancer cells.</td>
<td></td>
</tr>
<tr>
<td>Bio-chr.17cen probe was applied to CISH to confirm chromosome 17 polymorphism when 3-5 copies per nucleus in &gt;50% of cancer cells.</td>
<td>No amplification (Diploid or aneuploid)</td>
<td>TopoIIα copies &lt; chr.17cen probe copies and ≤6 copies per nucleus in &gt;50% of cancer cells.</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 1. CISH™ Detection Kit.* 
Fig. 2. CISH™ with SP Light HER2 probe, HER2 amplification, breast cancer, 40X.
Fig. 3. CISH™ with SP Light TopoIIα probe, TopoIIα amplification, breast cancer, 40X.
DISCUSSION

Our data confirm that TopoIIα aberration is common but only in HER2 amplified breast cancer. TopoIIα gene amplification or deletion were not found in breast cancers without HER2 amplification. Chromosome 17 centromeric probe is important to assist in assessing TopoIIα gene status (Fig. 4). TopoIIα amplification or deletion may clinically be highly relevant as indicated by earlier studies because TopoIIα is the molecular target for several important anti-cancer drugs, termed topoII inhibitors (Doxorubicin, Epirubicin...). So far, clinical studies have highlighted the role of HER2 as a predictive factor for topoII inhibitor chemotherapy and results are contradictory. Our results together with other studies encourage us to correlate the response to topoIIα inhibitors directly with TopoIIα gene amplification and deletion.

CONCLUSION

SPT™ DNA probe gives a highly specific and sensitive staining of TopoIIα gene. The cost of CISH probe is relatively low. Its ease of use in routine pathology laboratory provides a practical approach in the primary screening of TopoIIα gene status in tumor samples.

REFERENCES


RESULTS: SEE TABLE 2.

Table 2. Prevalence of TopoIIα amplification and deletion detected in 59 breast cancers according to HER2 amplification

<table>
<thead>
<tr>
<th>HER2 Gene Status</th>
<th>TopoIIα Gene Status</th>
<th>TopoIIα Amplification</th>
<th>TopoIIα Deletion</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Amplification (N=30)</td>
<td>30 (100%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Amplification (N=29)</td>
<td>13 (44.8%)</td>
<td>15 (51.7%)</td>
<td>1 (3.5%)</td>
</tr>
</tbody>
</table>

Fig. 4. Blocking Step

Blocking Solution

Blocking Solution

Ag DIG

Mouse Anti-DIG Ab

Ab

Polymerized HRP-Goat Anti-Mouse

Color Precipitate

Chromogen/Substrate HRP

Fig. 2. CISH™ Polymer Detection Kit

Fig. 3. CISH™ Centromere Detection Kit

Fig. 6a. CISH™ with SPOT: Light HER2 probe, HER2 amplification, breast cancer, 40X.

Fig. 6b. CISH™ with SPOT: Light TopoIIα probe, TopoIIα deletion, breast cancer, 40X. 2 dots/nucleus.

Fig. 6c. CISH™ with SPOT: Light Chromosome 17 centromeric probe, Chromosome 17, breast cancer, 40X. 3-5 dots/nucleus.