

Chromogenic in situ hybridization (CISH): A practical method to confirm the immunohistochemical (IHC) results for HER-2 amplification in archival breast carcinoma samples

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INTRODUCTION

Determination of HER-2 status has become necessary for selection of breast cancer patients for trastuzumab (Herceptin[®]) therapy. Trastuzumab is found to be effective only in patients whose tumors show amplified HER-2 gene and/or overexpressed HER-2 protein. Majority of HER-2 studies used IHC, which detects the HER-2 protein overexpression on the cell membrane. However, IHC is subjected to a number of technical artifacts and sensitivity differences between different antibodies and tissue pretreatments.

In order to accurately identify HER-2 status, we have developed CISH which uses HER-2 DNA probe produced by Subtracted Probe Technology (SPT[®]). With SPT technology, repetitive DNA sequences *Alu* and LINE elements, which cause non-specific hybridization, are quantitatively removed. Therefore, the final probe is very specific and blocking of non-specific hybridization with Cot-1 DNA in traditional FISH probe is eliminated. HER-2 probe from SPT technology gives sufficiently signals under bright field microscopy after enzymatic detection.

MATERIAL AND METHOD

Tumors: 63 breast cancer cases of formalin-fixed and paraffin-embedded (FFPE) samples were collected for this study.

CISH: CISH was done on 4mm tissue sections. Briefly, the section were de-paraffinized, followed by heat pretreatment in Pretreatment Buffer (SP●T-Light[™] Tissue Pretreatment Kit, Zymed) and pepsin digestion. Ready-to-use digoxigenin-labeled HER-2 probe (SP●T-Light[™] HER-2 probe, Zymed) was applied onto slides before the denaturation and hybridization. After the stringent wash, the HER-2 probe was detected with sequential incubation with FITC conjugated anti-digoxigenin antibody, HRP-conjugated anti-FITC antibody, and DAB according to the direction on manufacture's specification sheet (CISH Detection Kit, Zymed). Tissue sections were lightly counterstained with hematoxylin and cover-slipped.

Table 1: Interpretation of CISH results

High level amplification	>10 copies or large cluster of amplicon/nucleus in >50% of cancer cell.
Low level amplification	6-10 copies or small cluster of amplicon/nucleus in >50% of cancer cell. Biotin-labeled chromosome 17 centromeric probe (Zymed) was applied for CISH to confirm a low level of gene amplification.
Unaltered gene copy	1-5 copies/nucleus. If 3-5 copies/nucleus, biotin-labeled chromosome 17 centromeric probe (Zymed) was applied for CISH to confirm chromosomal aneuploidy.

IHC: IHC of HER-2 protein was done on 4mm tissue sections. Two monoclonal (TAB250, Zymed; and CB11, Novocastra) and two polyclonal antibodies (Hercep, Dako; Rb anti HER-2, Zymed) were used in this study. Tissue sections were deparaffinized followed by antigen-retrieval in citrate buffer (CB11, Hecept, Rb anti HER-2) or ficin pretreatment (TAB250). After blocking for non-specific antibody binding, primary antibody was added on the section and incubated for 1 hr at room temperature. NBA Kit (Non-biotin amplification system, Zymed) was used for visualization with DAB as the chromogen. The sections were counterstained with haematoxylin. Only membrane staining was considered as positive staining.

Table 2: Interpretation of IHC results

Score	HER2	Staining Pattern
3+	Positive	A strong complete membrane staining is observed in more than 10% of the tumor cells.
2+	Positive	A weak to moderate complete membrane staining is observed in more than 10% of the tumor cells.
1+	Negative	A faint/barely perceptible membrane staining is detected in more than 10% of the tumor cells. The cells may be only stained in part of the membrane.
—	Negative	No staining is observed, or membrane staining is less than 10% of the tumor cells.

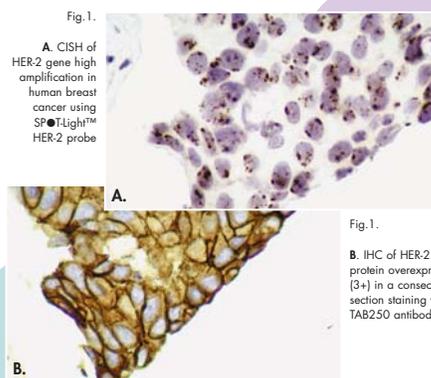


Fig. 1.

A. CISH of HER-2 gene high amplification in human breast cancer using SP●T-Light[™] HER-2 probe

B.

Fig. 1.

B. IHC of HER-2 protein overexpression (3+) in a consecutive section staining with TAB250 antibody.

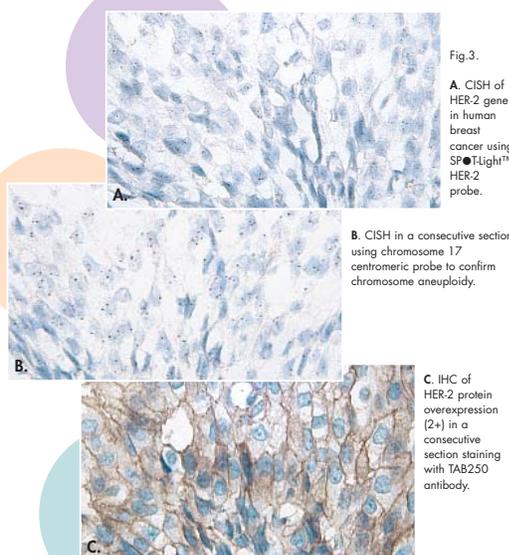


Fig. 3.

A. CISH of HER-2 gene in human breast cancer using SP●T-Light[™] HER-2 probe.

B. CISH in a consecutive section using chromosome 17 centromeric probe to confirm chromosome aneuploidy.

C. IHC of HER-2 protein overexpression (2+) in a consecutive section staining with TAB250 antibody.

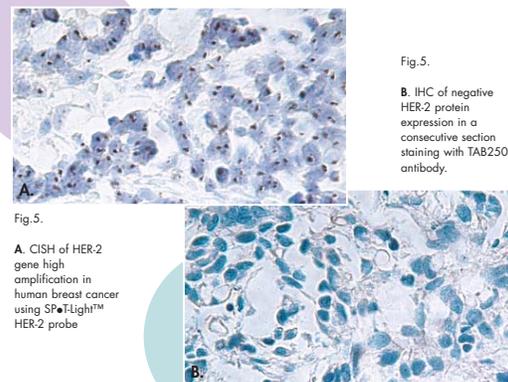


Fig. 5.

B. IHC of negative HER-2 protein expression in a consecutive section staining with TAB250 antibody.

Fig. 5.

A. CISH of HER-2 gene high amplification in human breast cancer using SP●T-Light[™] HER-2 probe

B.

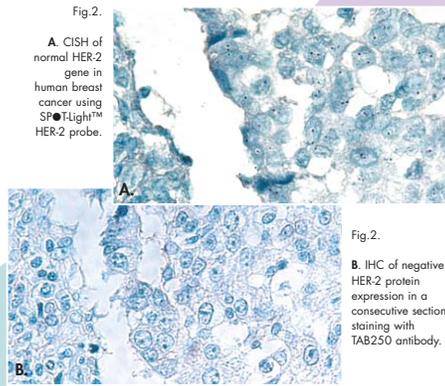


Fig.2.

A. CISH of normal HER-2 gene in human breast cancer using SPTLight™ HER-2 probe.

Fig.2.

B. IHC of negative HER-2 protein expression in a consecutive section staining with TAB250 antibody.

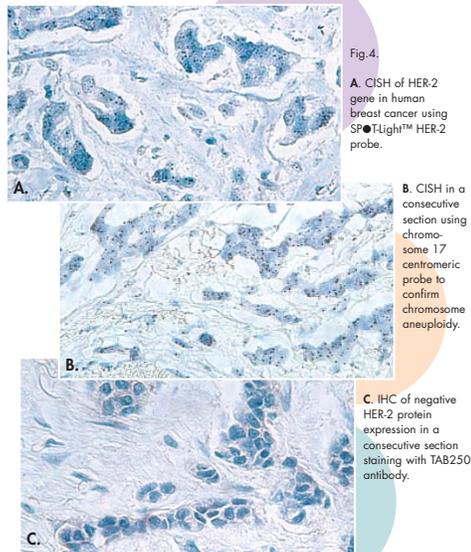


Fig.4.

A. CISH of HER-2 gene in human breast cancer using SPTLight™ HER-2 probe.

B. CISH in a consecutive section using chromosome 17 centromeric probe to confirm chromosome aneuploidy.

C. IHC of negative HER-2 protein expression in a consecutive section staining with TAB250 antibody.

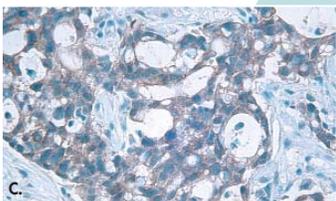


Fig.5.

C. IHC of negative HER-2 protein expression (1+) in a consecutive section staining with Hercep antibody.

RESULTS

Table 3: Comparison of CISH and IHC

CISH	IHC								
	TAB 250		CB11		Hercep		Rb HER2		
	3+/2+	1+/-	3+/2+	1+/-	3+/2+	1+/-	3+/2+	1+/-	
High (9) 14%	8	1*	8	1*	8	1**	8	1**	
Low (1) 2%	1	0	1	0	1	0	1	0	
Normal (53) 84%	1***	52	12	41	20	33	15	38	
16%	84%	16%	84%	33%	67%	46%	54%	38%	62%

* The sample is — for IHC (see Fig. 5.B.) ** The samples is 1+ for IHC (see Fig. 5.C.)

*** Samples is chromosome 17 aneuploidy (see Fig. 3.)

DISCUSSION

HER-2 probe generated by SPT is both specific and sensitive enough for peroxidase-base probe detection. CISH results can be analyzed more rapidly as compared to FISH slides, and verification of histopathology can be done simultaneously from the tissue section that is counterstained with hematoxylin, whereas the FISH does not allow sufficient histopathological evaluation of the cells. Additionally, CISH is less expensive and data is easier to store than FISH.

High gene amplification as detected by CISH correlates strongly with positive staining (3+/2+) for all four the antibodies except for one tissue with weak antibody staining (1+). Monoclonal antibody TAB250 gives the strongest correlation with CISH in cases with normal HER-2 status. The other 3 antibodies gives higher positive staining when CISH showed unaltered HER-2 gene.

In terms of feasibility and accuracy, HER-2 CISH provides a tempting alternative to HER-2 IHC, and CISH result is easier to interpret as it is more objective. With IHC, it is not possible to identify a false-negative staining, and it is not possible to discriminate whether a positive staining is due to HER-2 gene amplification, or amplification-unrelated HER-2 expression or non-specific staining. In CISH, the diagnosis of unaltered HER-2 gene status is based on the present of 1-5 gene copies in cancer cells. If CISH shows no hybridization signals, the failure is most likely due to technical reasons.

CONCLUSION

SPT DNA probe gives a highly specific and sensitive staining of HER-2 gene amplification. The cost of CISH probe is relatively low. It's easy to use in routine pathology laboratory provides a practical approach in the primary screening of HER-2 status in tumor samples, and it is useful in the confirmation of ambiguous IHC results.

REFERENCE

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