

Evaluation of MYC Gene Amplification in Prostate Cancer Using a Dual Color Chromogenic In Situ Hybridization (Dual CISH) Assay

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Abstract

Objective: The overall purpose of the study was to demonstrate applicability of the DAKO dual-color chromogenic in situ hybridization (CISH) assay (DAKO Denmark, Glostrup) with respect to fluorescence in situ hybridization (FISH) probes *MYC-C*.

Methods: *MYC* gene amplification by FISH and DAKO dual-color CISH

Results: The study showed that the dual-color CISH assay can convert Texas red and fluorescein isothiocyanate (FITC) signals into chromogenic signals. The dual-color CISH assay was performed on 40 cases of prostate cancer. Amplification was identified in 12 of 40 (30%) tumors. No amplification was seen in 28 of 40 (70%) tumors. FISH data were available in total of 40 tumors. All tumors showed concordant results between dual-color CISH and FISH for classifying a tumor as *MYC* amplified or not amplified.

Conclusions: We conclude that dual-color DAKO CISH assay is an accurate method for determining *MYC* gene amplification with added advantages that make it a more practically useful method.

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INTRODUCTION

Prostate cancer (PCa) is a common malignancy in men in Western countries, has the characteristics of a heterogeneous damage with multiple risk factors [1]. The increase in the incidence of PCa in the last twenty years has assumed almost epidemic proportions because this neoplasm is the most frequently diagnosed solid tumor and that in our country is the second leading cause of death oncology [2]. There is consistent evidence that PCa deaths have decreased over the last decade, most likely associated with the extensive use of prostate specific antigen (PSA) in the early diagnosis of the disease [3]. The 8q24.21 region, where MYC is located, is contained within a region that is commonly amplified in prostate cancer, especially in advanced and

recurrent disease [4]. By chromosome microdissection, 8q24 amplification was first identified in 2 prostate cancer cases [4]. To verify this, fluorescence in situ hybridization (FISH) was carried out on 44 prostatectomy samples, and the amplification was present in only 9 % of the total cases studied but 75 % of advanced cases. Accordingly, in the study of patients with recurrent disease, comparative genomic hybridization and FISH analysis showed 8q24 amplification in 8 of 9 cases [4].

Because the amplification of the 8q24 region is predominantly observed in late-stage/aggressive tumors, it has been widely held that MYC is involved in disease progression [4]. Interestingly, the amplification of MYC is generally on the order of a

few fold; high-level amplifications, such as those seen with MYC-N in a subset of neuroblastomas, are virtually never seen in prostate cancer.

Fluorescence in situ hybridization (FISH) has achieved widespread use for a range of gene copy number detection probes and translocation probes. It is considered a very accurate and sensitive method, e.g., the College of American Pathologists has published that FISH is to be regarded as “gold standard” for HER2 testing [5]. However, the FISH method is perceived as having some limitations. The evaluation of tumor morphologic features through FISH may be difficult, and the method requires a fluorescence microscope, which is costly and not readily available in all pathology laboratories.

Furthermore, the fluorescence signals fade relatively quickly, which makes archiving of the slides difficult. These limitations can be overcome by chromogenic in situ hybridization (CISH), which converts the fluorescence signals into chromogenic precipitates, and can visualize FISH-labeled probes along with the morphologic features using a brightfield microscope [6].

Hoff et.al [6] developed a dual-color CISH assay in which the green fluorescein isothiocyanate (FITC) FISH signals are converted into red chromogenic precipitates [7,8].

The purpose of this study was demonstrate the general use of the DAKO dual-color CISH assay (DAKO Denmark, Glostrup) by applying it to gene copy detection probe as *MYC-C*.

MATERIAL AND METHODS

Case Selection

Archival formalin-fixed paraffin-embedded tissues from 40 prostate cancers were randomly selected. An H&E stained section was evaluated for the presence of prostate cancer and used for assessment of MYC gene amplification by FISH and DAKO dual-color CISH.

Fluorescence In Situ Hybridization

The FISH analyzes for *MYC-C* were performed using FISH probes for C-MYC, respectively (DAKO) in combination with the Histology FISH Accessory kit (DAKO) (Code K5599). The FISH analyses for *MYC-C* were performed using the FISH (DAKO) assays. All staining were performed according to the respective package insert and subsequently evaluated using a fluorescence microscope. The slides that were analyzed using the dual-color CISH kit followed the same protocols but were not mounted.

Chromogenic In Situ Hybridization

The dual-color CISH (Code SK 108) assay contains reagents required to complete a 2-step immunohistochemical staining procedure to detect DAKO Texas red- and FITC-labeled FISH probes. First, the aforementioned FISH procedures were followed, except the last dehydration and mounting step was omitted and the tissue specimens for CISH analysis were immersed in the CISH Wash Buffer (DAKO). The next step in the CISH procedure is to block the tissue specimens for endogen peroxidase with a ready-to-use Peroxidase Blocker (DAKO). Peroxidase blocking is followed by incubation with a ready- to- use CISH Antibody Mix (DAKO), which comprises a mixture of anti-FITC conjugated with horseradish peroxidase and anti-Texas red conjugated with alkaline phosphatase. Tissue specimens were then incubated with a red chromogen followed by incubation with a blue chromogen [6]. Finally, the specimens were counterstained with hematoxylin (DAKO Hematoxylin - Code S3301) and coverslipped (Tissue-Mount, Aquatex de Merck), and the results were evaluated using a bright-field microscope.

Interpretation of Staining

Samples were scored according to the guidelines provided with the FISH probe used with the DAKO DuoCISH kit. However, the CISH signals are slightly larger than the corresponding FISH signals. For all tumor specimens in this study signals from 20 nuclei were counted.

RESULTS

In the forty patients studied, the average age was 63 years, with a median of 63.5 years, the youngest was 51 years and the largest 73. Twelve patients showed amplification of C-MYC gene, showing a prevalence of 30% of the sample.

The dual-color CISH assay was tested with MYC-C probe. This was qualitative, evaluating the general appearance of CISH staining with respect to tissue morphologic features and the balance between red and blue signal size, sharpness, and intensity. For the probe, the general appearance seemed almost identical between the 2 methods (**Figure 1**).

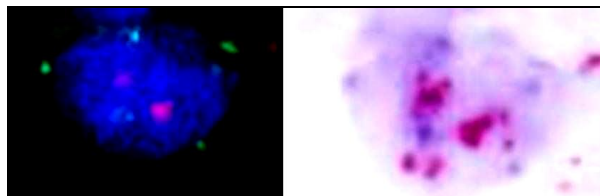


Figure 1. Dot-to-dot conversion of FISHsignals to CISH signals.

DISCUSSION

The present study showed that the dual-color CISH assay can convert Texas red and FITC FISH signals into chromogenic signals in context with preserved morphologic features. A number of previously published studies with HER2 probes have likewise shown high concordance between FISH and CISH assays, in the range of 91% to 100%, which seems to confirm the reliability of CISH [9-12].

The *MYC-C* sensitizes cells to a wide range of proapoptotic stimuli. During apoptosis, *MYC-C* induces the release of cytochrome c from mitochondria into the cytosol, possibly through the activation of proapoptotic molecule BAX. Similarly, other authors sought this type of alterations in prostate cancer. [13-15]. Pflueger et al [16], where they have identified NDRG1-ERG fusion in prostate tumors in 44% of patients (n = 100) who received radical prostatectomy, also considering patients with advanced disease. This identification of NDRG1-ERG fusion results in the formation of chimeric proteins in prostate cancer and has potential clinical and biological implications. The NDRG1 is involved in cell differentiation, suppressed by the oncogenes N-MYC and C-MYC and therefore often deregulated in cancer cells. It is expected that the testing of dual-color CISH in a relatively short time, is an accepted method in the routine evaluation of diagnosis of the situation in the different types of cancer. Using a light microscope instead of a fluorescence microscope and the ability to easily observe the morphological characteristics of the fund will be seen as advantages over current FISH assays. Based on data from different studies [6, 17, 18], our experience using the technique of CISH [19-23] and the results of this study, the dual-color DAKO CISH assay appears to be sensitive and specific, and because of his familiarity with immunohistochemical analysis, which may have the potential to be the most used in pathology laboratories in the future.

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CONFLICTS OF INTEREST

The authors declare that they have no conflict of interest.

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