

The use of Fluorescence in Situ Hybridization (FISH) in detecting ROS1 gene rearrangements in Non Small Cell Lung Cancer.

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ABSTRACT

Lung cancer is the second most common cancer with an estimated 220,000 new cases in 2011. CDC statistics also suggest that approximately 157,000 deaths will be caused by lung cancer which makes it more deadly than colon, breast and prostate cancers combined. About 85% of all lung cancer is categorized as non small cell lung cancer (NSCLC) including adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. There are tests available for a number of genes known to be involved in NSCLC tumorigenesis including EGFR, KRAS, BRAF, HER2, and ALK. Recently, ROS1 (c-ros oncogene 1, receptor tyrosine kinase) gene rearrangements have been identified in patients with NSCLC. Located on chromosome 6, ROS1 gene rearrangements have been found in 1-2% of all NSCLC patients. Rearrangements of the ROS1 gene leads to kinase activation and subsequent up-regulation in the cell proliferation pathway and reduced apoptosis. Recent literature suggests that Xalkori (crizotinib) a kinase inhibitor could be considered as a treatment for NSCLC patients that harbor ROS1 gene rearrangements. In January, 2013 ROS1 was mentioned in the NCCN guidelines under diagnostic and treatment options for patients with NSCLC. ROS1 has multiple rearrangement partners making a ROS1 break apart FISH probe set the most cost-effective strategy for identifying gene rearrangements in paraffin embedded tissues. We present our data on ROS1 gene rearrangements in NSCLC tumor samples. Of note is the observation that ROS1 shows similar FISH signal patterns, both normal and abnormal, to what has been reported in ALK-rearranged NSCLC tumors.

INTRODUCTION

Lung cancer is one of the leading causes of cancer deaths worldwide. Approximately 200,000 new cases were identified in 2011. The Centers for Disease Control and Prevention statistics suggests that over 157,000 lung cancer deaths will occur making lung cancers frequency higher than that of colon, breast and prostate cancer combined. Non small cell lung cancer (NSCLC) represents approximately 85% of all cases of lung cancer. NSCLC is represented by adenocarcinoma, squamous cell carcinoma, and large cell carcinoma. A number of genes are known to be involved in lung cancer tumorigenesis, including EGFR, KRAS, BRAF, HER2, and ALK. New research suggests that ROS1, formally known as c-ros oncogene 1, is a receptor tyrosine kinase located on chromosome 6 that is an oncogene when mutated/rearranged causes lung cancer. ROS1 gene rearrangements can be detected by FISH analysis and are reported at a frequency of 1-2% of known NSCLC cases. The method to detect ROS1 gene rearrangements involves hybridizing FISH probes that flank the ROS1 gene. This FISH test is performed on formalin fixed paraffin embedded (FFPE) lung tissue and allows the detection of the majority of ROS1 gene rearrangements reported in NSCLC. The ROS1 gene shares similar biologic activity with ALK, including predominant histology and patient demographics (young, never-smokers, increased frequency of Asians). Pre-clinical studies and early clinical reports show ROS1-rearranged tumors are sensitive to crizotinib treatment and demonstrates response rates comparable to ALK rearranged tumors. FISH testing has become crucial in the diagnosis of ROS1 positive NSCLC cases.



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Below are H&E stained tissue sections of lung histology. Figure 1 shows normal pulmonary aveoli (A) which are normally one to two cell layers thick. Within the normal lung are normal blood vessels (B). In NSCLC, specifically adenocarcinoma (Figure 2A) are found in the lung surrounded by connective tissue (Figure 2B).

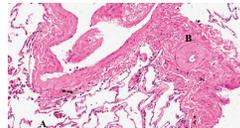


Fig. 1. Normal lung tissue using H&E stain

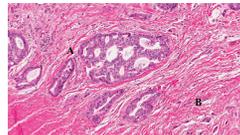


Fig. 2. Abnormal Lung tissue using H&E stain

MATERIALS & METHODS

Fluorescence in situ hybridization (FISH) was performed on formalin fixed paraffin embedded lung tissue sections that were cut 4-5 microns thick and placed on positively charged slides. The specimens used for this study were hybridized using a LDT for the ROS1 probe set and an FDA approved protocol for ALK probe. The region used to analyze the FISH signal pattern was identified by a pathologist and represents NSCLC (figure 2a) The ROS1 and ALK analysis results were compiled and evaluated. A comparison of ROS1 and ALK gene rearrangements was performed. Sample were considered positive if >50% of the first 50 cells scored are positive, and considered negative if <10% cells are positive. If 10-50% of cells are positive, an additional 50 cells are evaluated by a second technologist. The sample is then considered positive if >= 15% of all 100 cells scored are positive. A chart was formulated to show this assessment.

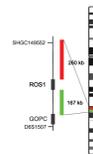


Fig. 3. Chromosome map of ROS1 probe set

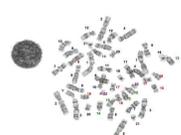


Fig. 4. G-banded Metaphase

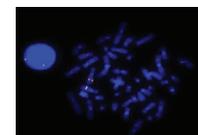


Fig. 5. Metaphase FISH with ROS1 probe set

RESULTS

Probe Validation for the ROS1 probe from MetaSystems, Allstutheim, Germany, was conducted on at least 15 normal specimens referred for adenocarcinoma or Non-Small Cell Lung Cancer to establish normal cutoff values. One normal specimen was run to confirm 100% sensitivity. Five metaphase spreads were probed and karyotyped with G- banding to confirm probe localization to 6q22 (Figure 3); a 375kb 5' segment distal to the ROS1 gene (GREEN signal) and a 370kb 3' segment proximal to the ROS1 gene (RED signal). Cutoff values for (3F or 1F) are calculated at 3 standard deviations from the average for each signal pattern observed.

The germline (non-rearranged; normal) signal pattern is two orange-green fusion signals (2F). The typical abnormal signal pattern includes one green, one orange and one green-orange fusion signal (1R1G1F) indicating a chromosome break in the ROS1 locus. Some variant or atypical abnormal signal patterns include 1R1F, 2R1G1F, 2R2G, ≥1R0G≥0F, and ≥1R0G≥1F.

We present our data collected from 100 different patients that ALK probe set and ROS1 probe set were performed on paraffin embedded lung tissue. This data shows how many cells were counts as normal (2F) or abnormal (1R1G1F, 1R1F, 1G1F, 1F, >=3F) in each probe set (see figures 6-8). For ROS1, the average number of 1F and 3F signal patterns were 8.27 and 13.8, respectively. For ALK, the average number of 1F pattern was 3.0 and the 3F signal pattern was 20.6. The number of samples that showed a hyperdiploidy pattern for both probe sets was 36%. Only 2 samples were confirmed positive for a ROS1 gene rearrangement and 3 were positive for ALK.

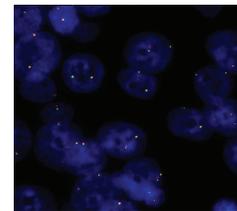


Fig. 6. Normal lung tissue hybridized with ROS1 probe set

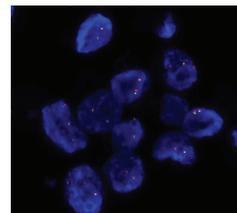


Fig. 7. Abnormal lung tissue hybridized with ROS1 probe set (3F)

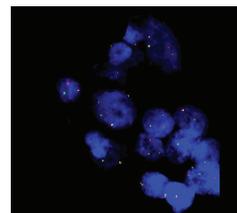


Fig. 8 Abnormal lung tissue hybridized with ROS1 probe set (1R1G1F, 1R1F, 1G1F)

DISCUSSION

Lung cancer is estimated to cause over 150,000 deaths and is the second most common cause of cancer. NSCLC represents about 85% of lung cancer cases and finding treatment for this condition is a medical necessity. Literature suggests patients with ALK or ROS1 rearrangements have an increased survival if treated with Xalkori (crizotinib), therefore, using FISH to identify tumors which may respond to this treatment is critical.

In this study, we report our ALK and ROS1 FISH analysis on tumor biopsies from 100 patients referred for NSCLC. Tissues were sectioned and hybridized using an FDA protocol for ALK and a laboratory developed test for ROS1.

We found a single fusion signal pattern in approximately 3% for ALK probe sets and 8% for ROS1 in our sample population. Approximately 20% of samples showed hyperdiploidy (>= 3F) for ALK and 14% had hyperdiploidy for ROS1. Of the 100 samples tested, 36% had hyperdiploidy for both ALK and ROS1. Approximately 26% had hyperdiploidy of at least ALK or ROS1. This would suggest that copy number gains in NSCLC is common.

For ALK, we found that 3 of 100 cases were positive for ALK gene rearrangements of greater than 15%, however we found seven (7) cases that had positive ALK rearrangements in 8-14% of nuclei analyzed. Because response to treatment using Xalkori (crizotinib) was only predicted to show response in patients with 15% rearrangements, patients within the 8-14% range were not eligible.

For ROS1, we found 2% of cases had greater than 15% gene rearrangements. Another 2 of 100 samples showed greater than 8% abnormality which would also prevent them from receiving Xalkori (crizotinib) treatment. None of the patient were positive for both ALK and ROS1 gene rearrangements, confirming that the rearrangements are mutually exclusive.

CONCLUSIONS

In this study of 100 cases of NSCLC cases referred for ALK and ROS1 testing, we found an abnormality rate of 3% and 2%, respectively. However, we found that rearrangements of 8% or greater was found in 7 samples for ALK and 2 for ROS1 which would exclude them from being eligible for Xalkori (crizotinib) treatment. Our data also suggests that hyperdiploidy is very high in NSCLC samples and the clinical significance of this finding is unclear. Further investigation of this finding is warranted.

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