Fluorescence *in situ* Hybridization (FISH) for 1p and 19q in Malignant Gliomas

Detection of Allelic Losses of the Short Arm of Chromosome 1 (1p) and Long Arm of Chromosome 19 (19q) by Fluorescence *in situ* Hybridization

Background

Malignant gliomas are the most common type of primary brain tumor and, historically, have been classified histologically as astrocytomas (including glioblastoma multiforme), oligodendrogliomas, ependymomas, and mixed gliomas. More recently, specific genetic alterations have been identified in gliomas that have been correlated with prognosis and response to therapy.

The majority of oligodendrogliomas show allelic loss of the short arm of chromosome 1 (1p). Patients with anaplastic oligodendrogliomas (WHO Grade III) having 1p loss have been reported to demonstrate better chemotherapeutic response and overall survival than those without 1p loss (Cairncross et al., 1998). Furthermore, combined loss of 1p and the long arm of chromosome 19 (19q) was shown to be a statistically significant predictor of prolonged survival in patients with oligodendrogliomas independent of tumor grade (Smith et al., 2000). Loss of 1p and 19q also occurs in a small percentage of high grade (WHO Grade III and IV) gliomas with astrocytic or mixed oligodendroglioma-astrocytoma histology. Retrospective analysis of these cases suggests a correlation between 1p/19q loss and outcome (Ino et al., 2000; Schmidt et al., 2002).

Clinical Indications

Cleveland Clinic Laboratories is now offering FISH analysis of chromosomes 1p and 19q to assist in treatment planning for patients with malignant gliomas. The results should be interpreted in the context of the tumor's clinical, radiographic, and histologic features, as well as any other known genetic information. As noted above, improved prognosis associated with 1p and 19q status has only been clearly demonstrated only in patients with oligodendroglioma histology.

Methodology

Fluorescent *in situ* hybridization (FISH) can be performed on all adequately fixed specimens, even those where the specimen is small in size or contains large areas of non-neoplastic cells in addition to representative tumor areas. The optimal fixative is 10% neutral buffered formalin. For each FISH analysis, a slide is hybridized with a target and a reference probe. Forty cells containing a minimum of two (2) reference probe signals are counted, and the ratio of target to reference probe signals is calculated.

1p FISH: LSI 1p36 / LSI 1q25 Dual Color Probe Set (Abbott Molecular, Vysis, Des Plaines, IL). This is comprised of a mixture of an ~ 400 kb SpectrumOrange labeled 1p36 target probe and an ~ 620 kb SpectrumGreen labeled 1q25 reference probe. The LSI 1p36 probe contains sequences that extend from near SHGC57243 locus, through the TP73 and EGFL3 genes and ends proximally at a point telomeric to the EGFL3 and RH75821 loci. The LSI 1q25 probe contains sequences that extend from a point telomeric to the WI-6848 locus, through the ABL2 and ANGPTL1 genes and ends proximally near the SHGC-1322 locus.

19q FISH: LSI 19p13 / LSI 19q13 Dual Color Probe Set (Abbott Molecular, Vysis, Des Plaines, IL). This is comprised of a mixture of an ~400 kb SpectrumOrange labeled 19q13 target probe and an ~500 kb SpectrumGreen labeled 19p13 reference probe. The LSI 19q13 probe contains sequences that extend from a point telomeric to the CRX locus, through the CRX, GLTSCR2 and GLTSCR1 genes and ends at a point centromeric to the GLTSCR1 locus by the RH76529 locus. The LSI 19p13 probe contains sequences that extend from a point centromeric to MAN2B1 locus, through MAN2B1, ZNF443 and ZNF44 genes and ends proximally at a point telomeric to the ZNF44 and RH92876 loci.

Interpretation

A target-to-reference probe signal ratio of <0.7 indicates loss of the target allele. A target-to-reference probe signal ratio >0.8 provides no evidence for allelic loss of the target locus. For specimens with ratios between 0.7 and 0.8, the test is repeated.

Loss of heterozygosity (LOH) analysis by PCR may be recommended in cases for which the FISH analysis is equivocal.

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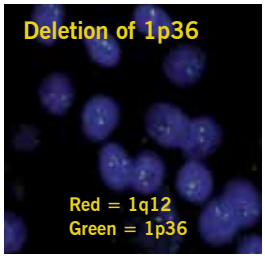
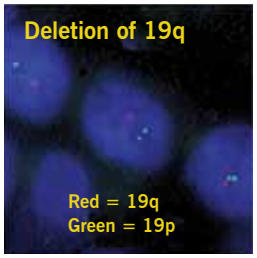
Limitations of the Assay

FISH examines only one locus for each chromosome. Allelic loss at one locus does not necessarily predict the presence of alterations at other loci on the same chromosome, i.e. FISH may not detect partial allelic loss. FISH may not detect allelic losses if there is loss of one allele and replication of the other allele. If more comprehensive locus-specific information is desired, we recommend requesting loss of heterozygosity (LOH) analysis by PCR in addition to FISH.





References

1. Cairncross JG, Ueki K, Zlatescu MC, *et al*. Specific genetic predictors of chemotherapeutic response and survival in patients with anaplastic oligodendrogliomas. *J Natl Cancer Inst*. 1998;90:1473-1479.
2. Ino Y, Zlatescu MC, Sasaki H, *et al*. Long survival and therapeutic responses in patients with histologically disparate high-grade gliomas demonstrating chromosome 1p loss. *J Neurosurg*. 2000;92:983-990.
3. Schmidt MC, Antweiler S, Urban N, *et al*. Impact of genotype and morphology on the prognosis of glioblastoma. *J Neuropathol Exp Neuro*. 2002;61:321-8.
4. Smith J. S, Perry A, Borell TJ, *et al*. Alterations of chromosome arms 1p and 19q as predictors of survival in oligodendrogliomas, astrocytomas, and mixed oligoastrocytomas. *J Clin Oncol*. 2000;18:636-645.

Test Overview

Test Name	FISH for 1p	FISH for 19q
Patient Preparation	None	None
Reference Range	Intact	Intact
Specimen Requirements	Formalin-fixed, paraffin -embedded tissue block from representative tumor or 10 serial, unstained, unbaked sections on positively charged slides. This is sufficient for both 1p and 19q FISH analyses. Paraffin block will be returned.	
Billing Code	81886	82271
CPT Code	88368 (x2)	88368 (x2)

Related Tests

Test Name	Billing Code
FISH for EGFR	82087
1p LOH by PCR	81883
TP53 Sequencing	82508

Technical Information Contact:

Wendy Nedlik MT(ASCP)
216.444.8410
nedlikw@ccf.org

Scientific Information Contacts:

Susan Staugatis, MD, PhD
216.444.9189
staugas@ccf.org

Richard Prayson, MD
216.444.8805
praysor@ccf.org