CystoSnap™ Bladder Cancer

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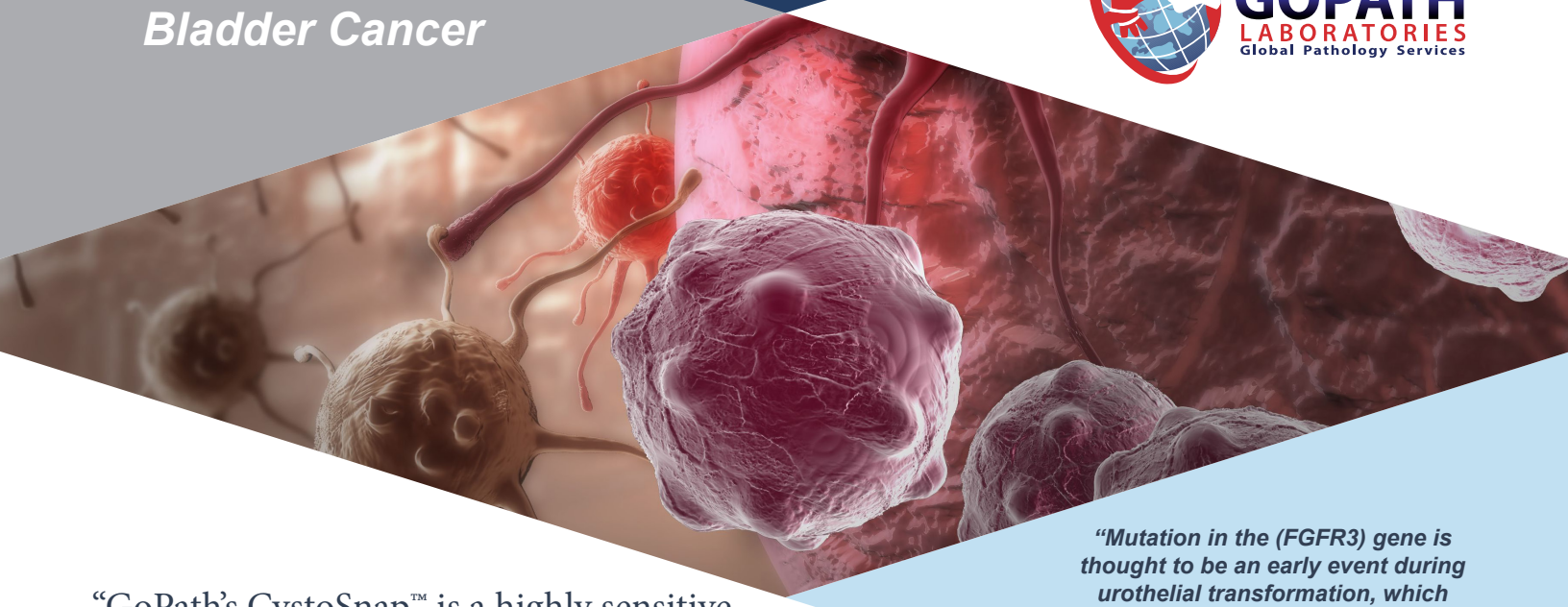
Global Pathology Services



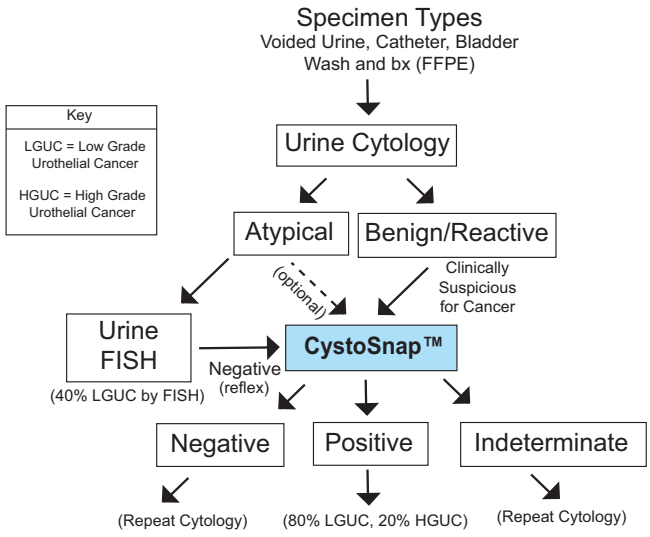
"Mutation in the (FGFR3) gene is thought to be an early event during urothelial transformation, which makes it the first marker that can be used for detection of bladder cancer in an early stage."

"GoPath's CystoSnap™ is a highly sensitive and specific test for bladder cancer"

Clinical Utility

Urothelial Carcinoma (UC) is the most common type of bladder cancer, primarily presenting as a non-muscle invasive tumor. Cystoscopy aided by cytology is the mainstay for the diagnosis and follow up of UC, but the sensitivity of cytology to detect low-grade UC (LGUC) is very low. Urine FISH assay increases the sensitivity for detection of UC, but is less specific and is still not optimal for LGUC. Fibroblast growth factor receptor (FGFR3) mutations commonly seen in LGUCs (~80%) are thought to be an early event during urothelial transformation, and can be detected in high-grade invasive UCs (~20%). Screening FGFR3 gene mutations at the initial diagnosis of urothelial carcinoma could potentially provide useful information for disease diagnosis and surveillance. Furthermore, patients with UC carrying FGFR3 mutations could potentially benefit from FGFR3-targeted therapies currently in development.

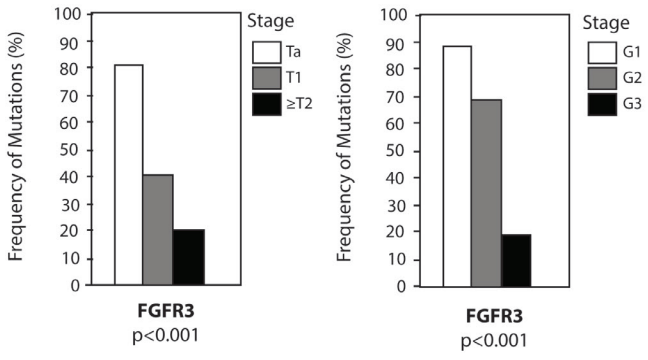
Figure 1. Algorithm For CystoSnap™



Assay Description and Methodology

DNA from urine cytology specimen (fresh cell pellet or cytology slide) or from formalin-fixed, paraffin-embedded tissue section is extracted using Pinpoint Slide DNA Isolation System™ (Zymo research). For each sample, one multiplex PCR reaction is performed to detect mutations at exon 7, 10, and 15 of the FGFR3 gene. After purification of the PCR product, a multiplex single nucleotide primer extension is then performed using the SNaPshot Multiplex kit reagents (Applied Biosystems, Foster City, CA). The fragments are separated by capillary electrophoresis and then analyzed. The limit of detection for the assay is about 3-5 percent.

Figure 2. Frequencies of FGFR3 mutations according to stage and grade*

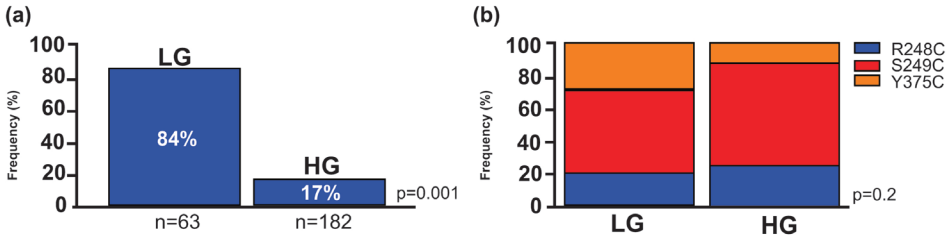


* Figure 2. is adapted and modified to show only the FGFR3 gene

Kompier LC, LurAkin I, van der Aa MNM, van Rhijn BWG, van der Kwast TH, et al. (2010) FGFR3, HRAS, KRAS, NRAS and PIK3CA Mutations in Bladder Cancer and Their Potential as Biomarkers for Surveillance and Therapy. PLoS ONE 5(11): e13821. doi:10.1371/journal.pone.0013821

http://journals.plos.org/plosone/article?id=info:doi/10.1371/journal.pone.0013821

Figure 3. Incidence and distribution of FGFR-3 mutations in low / high-grade UC

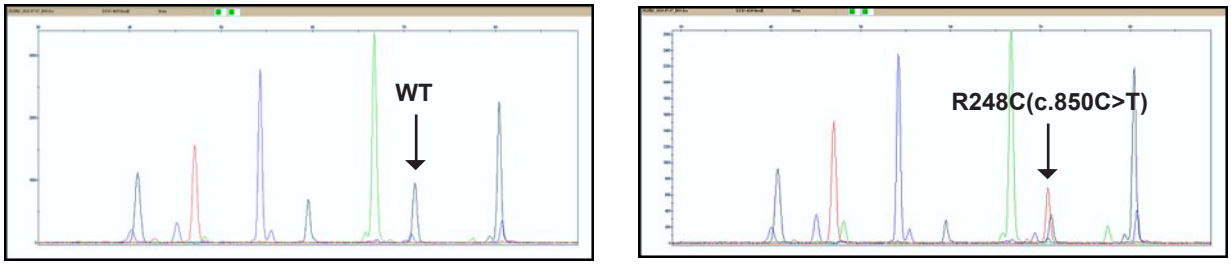


Al-Ahmadie HA, Iyer G, Janakiraman M, Lin O. Somatic mutation of fibroblast growth factor receptor-3 (FGFR3) defines a distinct morphological subtype of high-grade urothelial carcinoma. J Pathol. 2011 Jun;224(2):270-9.

Interpretation

Activating mutations in the fibroblast growth factor receptor 3 (FGFR3) are the most common and specific genetic alterations in bladder cancer. The mutations are frequently seen in non-muscle-invasive and low-grade urothelial carcinomas as well as in some high-grade urothelial carcinomas. Mutation in the FGFR3 gene is thought to be an early event during urothelial transformation, which makes it the first marker that can be used for detection of non-muscle-invasive bladder tumors. Currently, FGFR inhibitors for solid tumors are in clinical trials. See Figure 4 for an example of a positive gene mutation interpretation in FGFR3.

Figure 4. FGFR3 Mutation Detection by SNaPshot Analysis



Samples For Submission

Urine cytology samples: Bladder and urethral washings, catheterized urine, voided urine, FISH urine samples, urine cytology slide, urinary tract biopsy samples, or FFPE sections.

Inadequate Samples

When squamous cell contamination in the urine specimen is greater than 90% of the total cells, it is considered inadequate. At least 10% or greater of the total should be urothelial cells.

Assay Limitations

1. Sensitivity of the assay established by our lab for detection of FGFR3 mutations is 3-5%.
2. Detection of a mutation indicates the presence of tumor cells in the tested urine sample. A negative result can only indicate that either no tumor cells were in the tested urine sample or the percentage of tumor cells is below the sensitivity cutoff.
3. Due to the nature of the tumor, not all urothelial carcinomas harbor FGFR3 mutations. The negativity does not exclude the possibility of a tumor with wild type FGFR3 present in the urinary tract. Therefore, combination with urine cytology can increase the sensitivity of detection.

References

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