

A New RAS G12D-Specific Rabbit Polyclonal Antibody for Immunohistochemical FFPE Application

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Background

Determining RAS mutation status is considered a pivotal factor for predicting the utility of anti-epidermal growth factor receptor (EGFR) antibody therapy in advanced colorectal cancer patients [1]. Because genetic testing remains costly and time consuming, it is necessary to develop mutation-specific antibody for immunological analysis. Thus, a reliable RAS G12D mutation antibody validated for immunohistochemical (IHC) staining would be a very useful reagent.

Aim

To develop novel RAS G12D mutation detection antibody for IHC staining

Results

Here we report a novel RAS G12D rabbit polyclonal antibody (GTX132407) validated IHC testing, developed in a cooperative effort with GeneTex, Inc. Based on the analysis of fifteen clinical colorectal cancer specimens, the results of IHC staining using the RAS G12D mutation-specific antibody (GTX132407) correlated well with genetic detection of the RAS G12D mutation using Ultra Sensitive Total RAS Mutation Screen Kit. [Hong-Jing Biotechnology, Inc. (FemtoPath)].

Material and Method

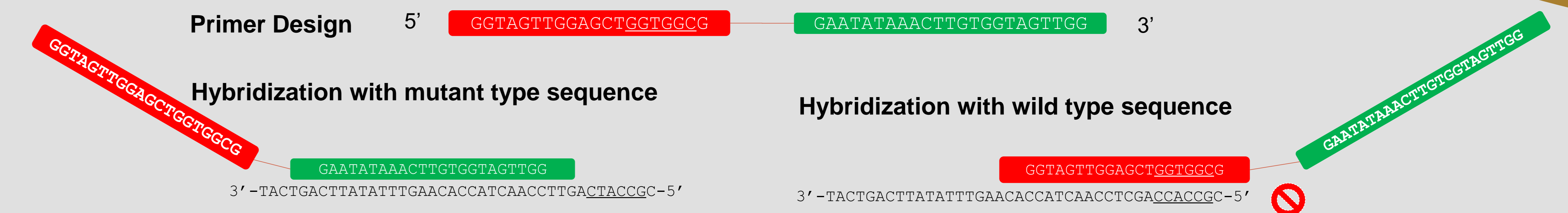
Genetic testing:

The Qiagen DNA Extraction Kit (QIAamp DNA FFPE Tissue Kit, Cat. No. 56404) was used for genomic DNA extraction. The Ultra Sensitive Total RAS Mutation Screen Kit is PCR-based and uses novel and proprietary primers which can specifically and sensitively amplify mutated sequences and inhibit wild type sequence amplification. Mutation Screen Kits from FemtoPath enable the sensitive detection of less than 5% mutated genes mixed with wild type sequence beginning with just 20~100 ng of DNA.

Immunohistochemistry:

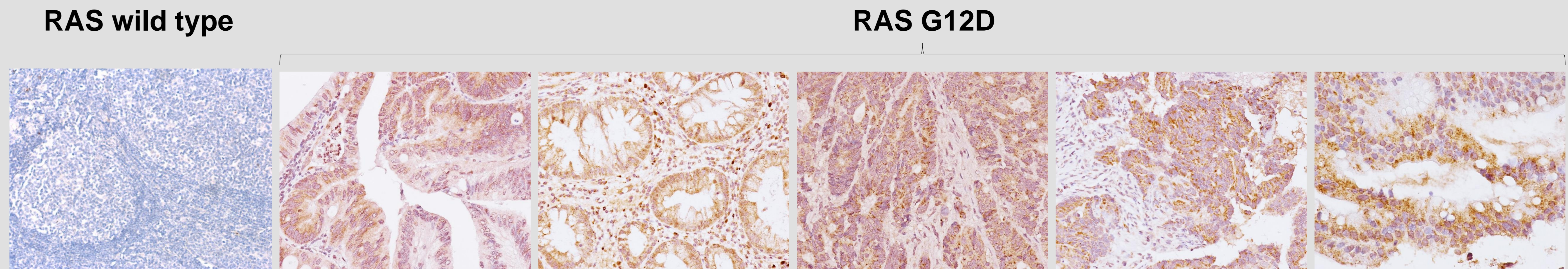
Formalin-fixed, paraffin-embedded (FFPE) clinical colorectal cancer tissue specimens with the RAS G12D mutation were analyzed with the FemtoPath (Patent No. P2708-TW) Ultra Sensitive Total RAS Mutation Screen Kit. The RAS G12D (GTX132407, GeneTex, Inc.) mutation detection antibody was applied on a BenchMark XT fully automated IHC/ISH staining instrument using the OptiView DAB IHC Detection Kit (Ventana Medical Systems, Inc.).

Schematic diagram of Ultra Sensitive Total RAS Mutation Screen Kit



IHC Results

RAS G12D rabbit polyclonal antibody (GTX132407) staining of human colorectal cancer tissue specimens.



Conclusions

Comparing the results with sequencing data from Femtopath, it shows that GeneTex's RAS G12D-specific mutation detection antibody could be a reliable tool for predicting RAS genetic mutation status by IHC.

References

1. Biomarkers predicting resistance to epidermal growth factor receptor-targeted therapy in metastatic colorectal cancer with wild-type KRAS. *Onco Targets Ther.* 2016 Jan 27;9:557-65.