Can immunohistochemistry detect KRAS G12D mutations in colorectal carcinoma?

Ana Raquel Lobo Da Rocha

York and Scarborough Teaching Hospitals NHS Foundation Trust, Wigginton Rd,York YO31 8HE

KEYWORDS

Colorectal cancer, Immunohistochemistry (IHC), antibody, G12D KRAS mutation

INTRODUCTION

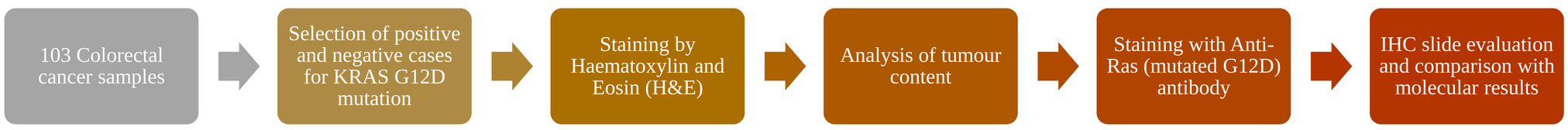
In the United Kingdom, colorectal cancer has a mortality rate of 6.7%, being the second most common cause of cancer death (IARC, 2020).
Patients diagnosed with metastatic colorectal cancer can receive anti–epidermal growth factor receptor (EGFR) targeted therapy but their response to the treatment depends on the mutational status of Rat sarcoma virus (RAS) and v-Raf murine sarcoma viral oncogene homolog B1 (BRAF) genes (Zhou, Ji and Li, 2021). The most frequent Kirsten RAS (KRAS) mutation in colorectal carcinoma is G12D, followed by G12V and G13D (Meng et al., 2021).

Currently, molecular techniques are the reference standard method to detect the RAS and BRAF mutations in colorectal cancer. These techniques are expensive and require specialised equipment and staff. Most of the Histopathology departments do not have a molecular laboratory, and therefore, they need to refer work to reference centres for molecular diagnostic tests increasing costs and turnaround times. However, immunohistochemistry is present in most of the Histopathology laboratories; it does not require additional equipment or expertise and it has a shorter turnaround time.

AIM

Evaluate if immunohistochemistry can detect the most frequent mutation in KRAS (G12D) as an alternative to molecular testing in colorectal cancer.

METHOD



RESULTS

In the positive control, it was expected to have antibody staining of malignant cells, but no staining occurred, with exception of non-specific staining of mucin in the normal epithelium of the colon. In the negative control, it was expected to have no staining in any of the tissue components, however mucin in the normal epithelium was also stained.
In several cases, this antibody stained some cells of normal epithelium and some cells of the inflammatory infiltrate. It was noticed that in some cases this staining was strong and crisp supporting a true staining (Figure 1C and 1D). No tumour cells were stained in any of the slides with exception of one case that showed partial cytoplasmatic staining of malignant cells (Figure 2). Since the molecular result for this case was negative and none of the other positive cases (by molecular techniques) stained positive with this antibody, this staining is likely to be a false positive.

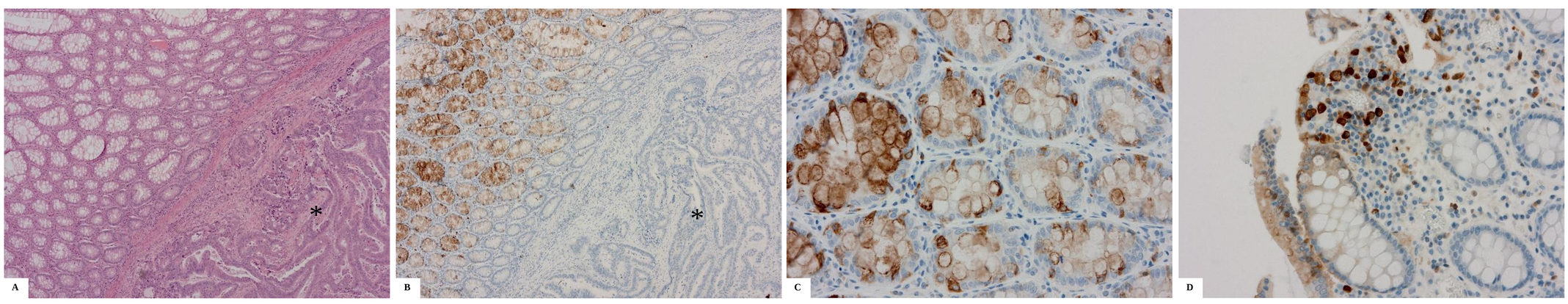


Figure 1: Case 85, negative for KRAS G12D mutation by molecular techniques. **A:** Area containing malignant cells of colon tumour (marked with ***)** and non-neoplastic colon epithelium stained by H&E, Magnification 40x. **B:** Area containing malignant cells of colon tumour (marked with ***)** and non-neoplastic colon epithelium stained with rabbit polyclonal anti-K-Ras G12D antibody, Magnification 40x. **C:** Non-neoplastic colon epithelium positively stained with rabbit polyclonal anti-K-Ras G12D antibody, Magnification 200x. **D:** Cells of the inflammatory infiltrate positively stained with rabbit polyclonal anti-K-Ras G12D antibody, Magnification 200x.

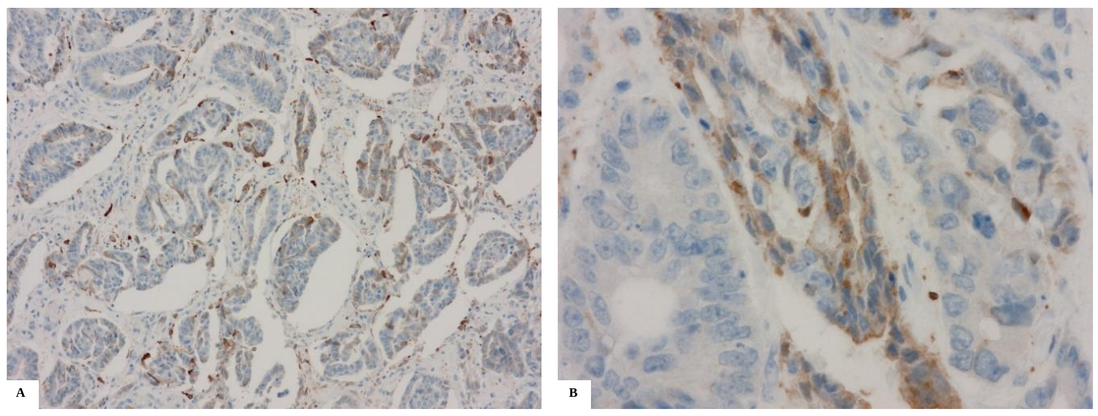


Figure 2: Case 100, negative for K-RAS G12D mutation by molecular techniques but stained positive with rabbit polyclonal anti-K-Ras G12D antibody. **A:** Focal cytoplasmic staining of malignant cells, Magnification of 100x. **B:** Colorectal tumour cells staining positive, Magnification of 400x.

DISCUSSION/CONCLUSION

Factors that could explain the lack of staining of the K-Ras G12D mutated protein:

- Sensitivity and specificity of the antibody,
- Batch-to batch variability (monoclonal vs polyclonal antibodies),
- Delay and length of specimen fixation,
- Tissue-specific factors,
- Choice of automated immunostaining platform.

The antibody used in this study failed in detecting the presence of K-Ras G12D protein in all the known positive cases for KRAS G12D mutations. Thus, IHC is not a reliable alternative to molecular techniques in detecting KRAS G12D mutations in CRC.

References

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