THE PYROSEQUENCING EXPERIENCE: TESTING COLORECTAL CANCER PATIENTS FOR KRAS STATUS IN THE CLINICAL PRACTICE

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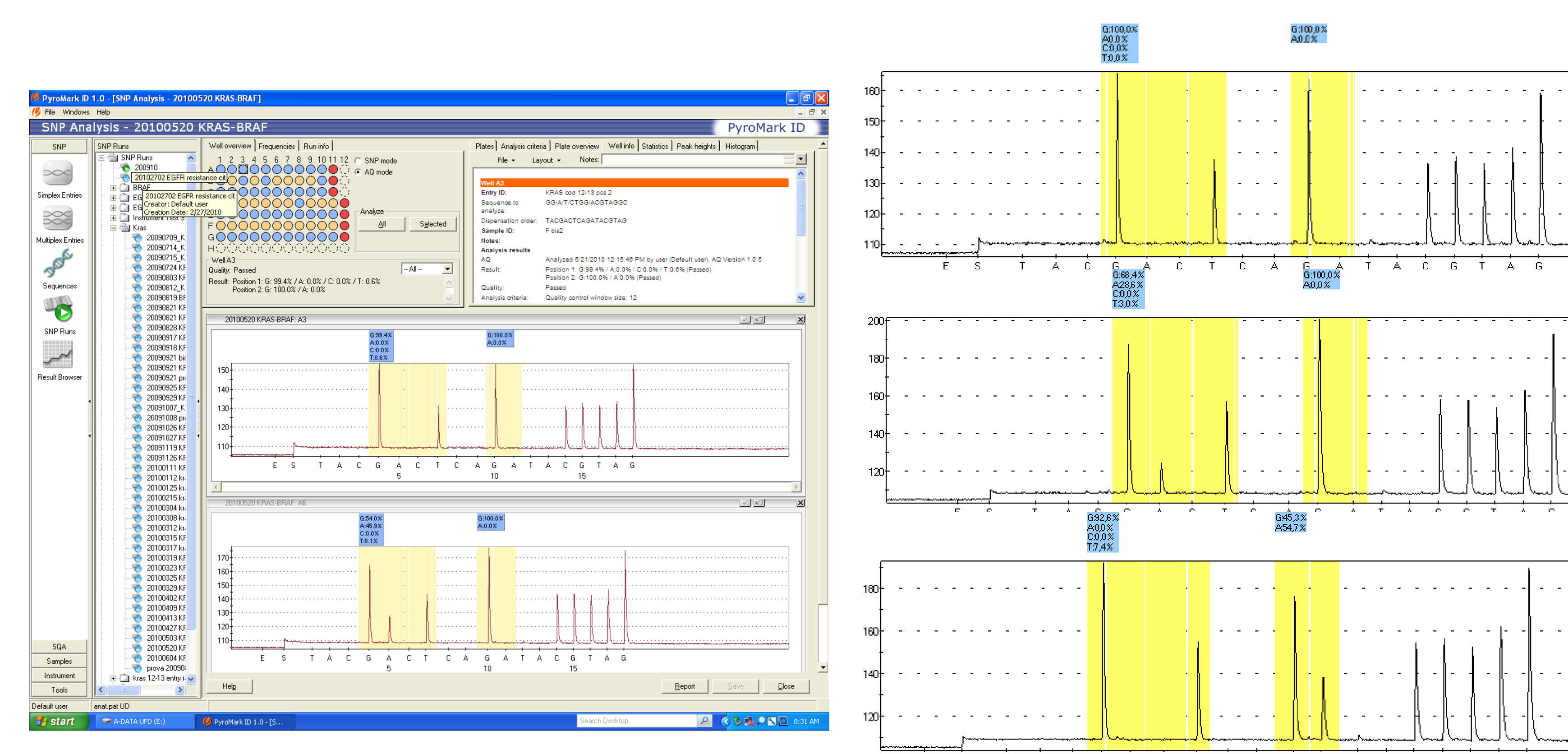
BACKGROUND

Testing KRAS status in advanced colorectal cancers has gained a practice-changing level of evidence, being predictive of resistance to the point of rendering the use of EGFR-inhibitors useless in patients carrying KRAS mutations. Since there is no agreement on the best testing method, different assays are commonly used in the clinical practice to determinate KRAS status. Among others, real-time PCR, direct sequencing analysis, and PCR-RFLP are the most widely used in Italy, while <10% of laboratories use pyrosequencing technology, a nucleotide extension based sequencing technique. Most of KRAS mutation tests are laboratory based. Limited information has been published on the application of CE-marked commercial kits with pyrosequencing.

Aims of our study were to verify the concordance of pyrosequencing technique with a CE-marked kit for clinical use with literature data and to validate the efficiency of this methodology in a consecutive series of 122 advanced CRC patients.

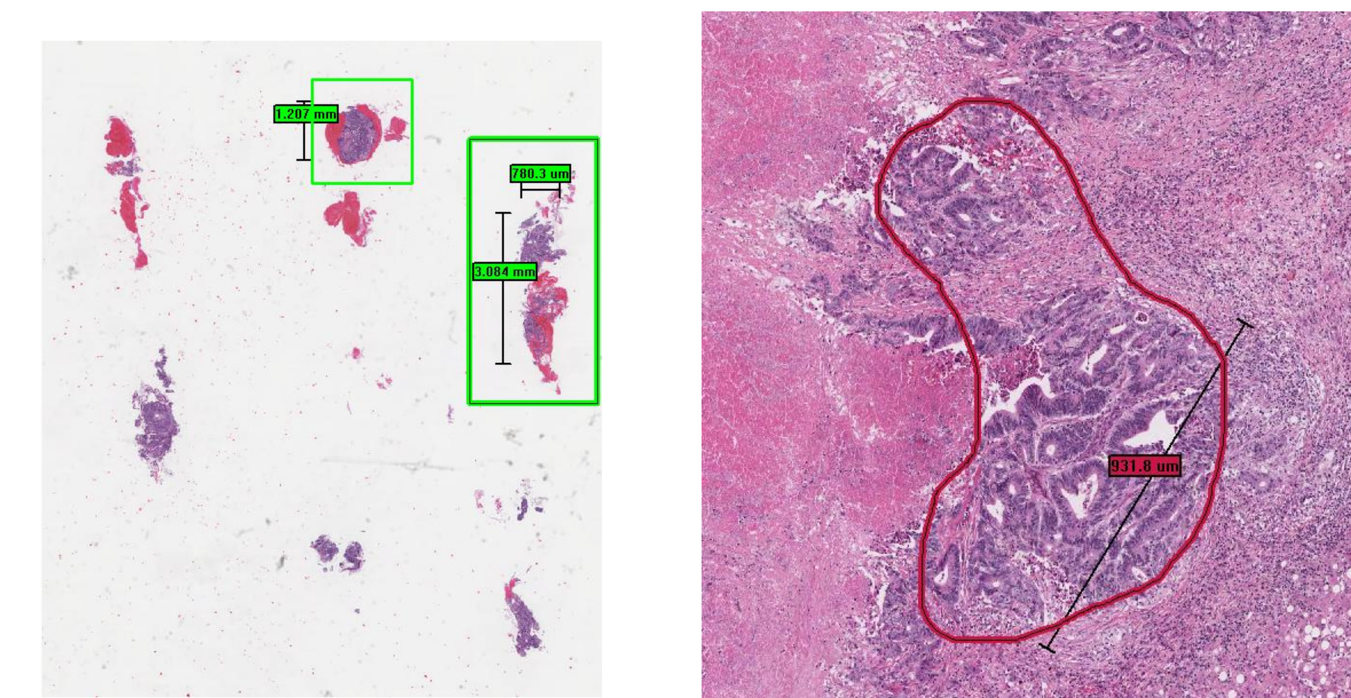


METHODS

Formalin-fixed and paraffin-embedded samples of 122 advanced colorectal carcinomas were retrieved from the archive of the Pathology Department. Almost 30% of all samples were small biopsies: infiltrates of tumor <6mm^2, percutaneous liver biopsies or endoscopic colonic biopsies. In 31 (25%) cases both primary tumor and corresponding metastases were analyzed. Histological slides were reviewed in order to guarantee an accurate selection of tumor areas and tumor microdissection was performed warranting a minimum of 70% of cancer versus non-tumor cells. DNA extraction was performed using QIAamp DNA Mini kit (Qiagen, Germany). Assays of tissue samples for KRAS and BRAF mutations were performed using Anti-EGFR MoAb response® (KRAS status) (Diatech, Italy) and Anti-EGFR MoAb response® (BRAF status) (Diatech, Italy), respectively, according to manufacturer's instructions. DNA was amplified by PCR reactions on Rotor-GeneTM 6000 (Corbett Research, Australia), single-stranded DNA templates were prepared using the PyroMark Vacuum Prep Workstation (Biotage, Sweden), and pyrosequencing analysis was finally performed on PyroMarkTM Q96 ID instrument (Biotage, Sweden).

RESULTS

Among 122 tested tumors, 60 (49%) harbored KRAS mutations. In particular, 41 patients reported a mutation on codon 12 (G12V, 12 pts; G12D 15 pts; G12C 5 pts; G12S 4 pts; G12R 1 pt; G12A 4 pts; c34_35GG>TT 1 pt); 13 patients on codon 13 (G13D); 2 patients on codon 61 (Q61H), and 4 patients on codon 146 (A146T). Among KRAS wild-type cases, we also found 4 patients with tumors bearing mutated BRAF (exon 15, V600E). No concurrent mutation on KRAS and BRAF was found. We reported an absolute concordance (100%) for KRAS status when analyses were performed both on primary tumors and on corresponding metastases.



CONCLUSION

Pyrosequencing is a promising, highly sensitive method, and the widespread availability of CE-marked kits to test KRAS and BRAF status would improve their implementation in routine practice. Data obtained is robust and confirms frequencies and pattern of mutations reported in the literature. Notably, the possibility to accurately determine KRAS status even in limited tissue samples obtained from small biopsies is key in the clinical practice. The slightly higher reported rate of KRAS mutations may reflect the high sensibility of the method.

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