Targeted KRAS mutation assessment on patient tumor histologic material in real time diagnostics.

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Source

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Abstract

BACKGROUND:

Testing for tumor specific mutations on routine formalin-fixed paraffin-embedded (FFPE) tissues may predict response to treatment in Medical Oncology and has already entered diagnostics, with KRAS mutation assessment as a paradigm. The highly sensitive real time PCR (Q-PCR) methods developed for this purpose are usually standardized under optimal template conditions. In routine diagnostics, however, suboptimal templates pose the challenge. Herein, we addressed the applicability of sequencing and two Q-PCR methods on prospectively assessed diagnostic cases for KRAS mutations.

METHODOLOGY/PRINCIPAL FINDINGS:

Tumor FFPE-DNA from 135 diagnostic and 75 low-quality control samples was obtained upon macrodissection, tested for fragmentation and assessed for KRAS mutations with dideoxy-sequencing and with two Q-PCR methods (Taqman-minor-groove-binder [TMGB] probes and DxS-KRAS-IVD). Samples with relatively well preserved DNA could be accurately analyzed with sequencing, while Q-PCR methods yielded informative results even in cases with very fragmented DNA ($p<0.0001$) with 100% sensitivity and specificity vs each other. However, Q-PCR efficiency (Ct values) also depended on DNA-fragmentation ($p<0.0001$). Q-PCR methods were sensitive to detect<or=1% mutant cells, provided that samples yielded cycle thresholds (Ct)<29, but this condition was met in only 38.5% of diagnostic samples. In comparison, FFPE samples (>99%) could accurately be analyzed at a sensitivity level of 10% (external validation of TMGB results). DNA quality and tumor cell content were the main reasons for discrepant sequencing/Q-PCR results (1.5%).

CONCLUSIONS/SIGNIFICANCE:

Diagnostic targeted mutation assessment on FFPE-DNA is very efficient with Q-PCR methods in comparison to dideoxy-sequencing. However, DNA fragmentation/amplification capacity and tumor DNA content must be considered for the interpretation of Q-PCR results in order to provide accurate information for clinical decision making.