Adaptation of a RAS pathway activation signature from FF to FFPE tissues in colorectal cancer

Bernard Omolo, Mingli Yang, Fang Yin Lo, Michael J. Schell, Sharon Austin, Kellie Howard, Anup Madan and Timothy J. Yeatman

Abstract

Background: The KRAS gene is mutated in about 40 % of colorectal cancer (CRC) cases, which has been clinically validated as a predictive mutational marker of intrinsic resistance to anti-EGFR inhibitor (EGFRi) therapy. Since nearly 60 % of patients with a wild type KRAS fail to respond to EGFRicombination therapies, there is a need to develop more reliable molecular signatures to better predict response. Here we address the challenge of adapting a gene expression signature predictive of RAS pathway activation, created using fresh frozen (FF) tissues, for use with more widely available formalinfixed paraffin-embedded (FFPE) tissues. Methods: In this study, we evaluated the translation of an 18-gene RAS pathway signature score from FF to FFPE in 54 CRC cases, using a head-to- head comparison of five technology platforms. FFPE-based technologies included the Affymetrix Gene Chip (Affy), NanoString nCounter™ (NanoS),Illumina whole genome RNASeq (RNA-Acc), Illumina targeted RNASeq (t-RNA), and Illuminastranded Total RNA-rRNA- depletion (rRNA).

Results: Using Affy_FF as the “gold” standard, initial analysis of the 18-gene RAS scores on all 54samples shows varying pairwise Spearman correlations, with (1) Affy_FFPE (r= 0.233, p = 0.090); (2)NanoS_FFPE (r= 0.608, p < 0.0001); (3) RNA-Acc_FFPE (r= 0.175, p = 0.21); (4) t-RNA_FFPE (r=−0.237, p = 0.085); (5) and t-RNA (r= −0.012, p = 0.93). These results suggest that only NanoString has successful FF to FFPE translation. The subsequentremoval of identified “problematic” samples (n= 15) and genes (n= 2) further improves the correlations of Affy_FF with three of the five technologies: Affy_FFPE (r= 0.672, p < 0.0001); NanoS_FFPE (r= 0.738, p < 0.0001); and RNA-Acc_FFPE (r= 0.483, p = 0.002).

Conclusions: Of the five technology platforms tested, Nano String technology provides a more faithful translation of the RAS pathway gene expression signature from FF to FFPE than the Affymetrix GeneChip and multiple RNASeq technologies. Moreover, Nano String was the most forgiving technology in the analysis of samples with presumably poor RNA quality. Using this approach, the RAS signature score may now be reasonably applied to FFPE clinical samples.