MultiOmix™: A multiplexed immunofluorescent assay capable of profiling protein expression and phosphorylation, in combination with next-generation sequencing from a single FFPE tissue section

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A comprehensive signaling pathway profile in combination with mutational analysis may be a critical guide for selecting effective clinical strategies for targeted drugs in combinations or in sequential regimens. In the United States, colorectal cancer (CRC) is the first most common cancer and the third leading cause of tumor associated death in men and women. CRC is a heterogeneous disease defined by different receptor tyrosine kinase (RTK) activation, signaling through phosphatidylinositol 3-kinase (PI3K)/AKT and RAS/MAP2K pathways. In addition, activating mutations in RTK and/or activating or loss-of-function mutations in downstream intracellular signaling proteins, can alter the efficacy of targeted drugs resulting in an ineffective treatment.

The Ion AmpliSeq™ cancer panel, consisting of 50 targeted genes, was used to perform a mutational analysis on each tumor. This panel contains a large number of well-known genes including EGFR, HER2, HER3, and cMET that play a critical role in CRC. The pipeline was designed to perform both protein expression/activation and mutational profiling from NGS. The data demonstrates concordance of both EGFR and HER2 expression correlated with positive staining for phospho-ERK1/2 and phospho-AKT, possibly through EGFR:HER2 and HER2:HER3 dimers, respectively. In tumor 2, mutational profiling revealed KRAS, G 12V (c.35G>T), known to activate the RAS-MAP2K pathway and activation of pERK1/2. In tumor 3, mutational profiling revealed PIK3CA, E545K (c.1635G>A), known to activate the PI3K-AKT pathway and activation of pAKT, and loss of PTEN protein which acts as a tumor suppressor to dephosphorylate PIP3 to PIP2 correlated with positive staining for phospho-ERK1/2 and phospho-AKT, possibly through HER2:HER3 dimer. In tumor 4, loss of PTEN protein which acts as a tumor suppressor to dephosphorylate PIP3 to PIP2, correlated with pAKT activation, possibly through upstream RTKs or intracellular signaling proteins not profiled in this study. Normal EGFR copy number were detected across all four tumor samples shown.

Mutational analysis was then performed on tumor and tumor adjacent regions, and sequenced using the Ion AmpliSeq™ cancer panel, consisting of 50 targeted genes.

Overview of MultiOmxy Technology Workflow

1. Capture-DNA
   2. RNA-seq/Exome
   3. Digital Spatial Analytics

Comparison of MultiOmxx Multiplexed IF to IHC

Figure 3. Comparison of MultiOmxx Multiplexed IF to IHC. The top row illustrates "Molecular" DAB or mDAB, which were generated from the IF images (data not shown), and demonstrates equivalent staining to the standard IHC. DAB images in CRC, IHC.

Analysis of RTK with Multiplexed IF and IHC

Figure 4. Characterizations of RTKs in CRC by MultiOmxx Multiplexed IF and IHC. The key RTKs (EGFR, HER2, HER3 and cMET) were characterized in CRC tumors by MultiOmxx Multiplexed IF staining. The EGFR IF stain was performed after the completion of multiplexed IF staining on the same slide. Four distinctive patterns of biomarker expression are shown, indicating activation of different signaling pathway. MultiOmxx multiplexed profiling can categorize CRC into different subtypes.

Integrated Analysis

Figure 5. Heterogeneous protein expression/activation and mutational profiling. In tumor 1, strong HER2 expression correlated with activation of pAKT, possibly through HER2:HER3 dimers. In tumor 2, expression of both EGFR and HER2 in presence of HER2 correlated with positive staining for phospho-ERK1/2 and phospho-AKT, possibly through EGFR:HER2 and HER2:HER3 dimers, respectively. In tumor 3, mutational profiling revealed KRAS, G 12V (c.35G>T), known to activate the RAS-MAP2K pathway and activation of pERK1/2. In tumor 4, loss of PTEN protein which acts as a tumor suppressor to dephosphorylate PIP3 to PIP2, correlated with pAKT activation, possibly through upstream RTKs or intracellular signaling proteins not profiled in this study. Normal EGFR copy number were detected across all four tumor samples shown.

Conclusion

MultiOmxx is capable of identifying extracellular Tumor Mutations through combined analysis of both IF protein expression/activation and mutational profiling from NGS. The data demonstrates concordance between upstream RTK expression and pathway activation with mutations identified in the AmpliSeq panel. Additionally, immunophenotyping revealed differences in lymphocytes infiltration and levels of immune infiltration has been shown in literature to correlate with clinical outcome. A comprehensive pathway signaling and immune profiling, combined with mutational analysis, may be necessary to select patients who may benefit from a single or combination targeted therapy tailored to their individual tumor profile.

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