KRAS Gene Amplification Defines A Distinct Molecular Subgroup of Gastroesophageal Adenocarcinoma

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Abstract

• The incidence of KRAS mutations in gastroesophageal cancer (GEC) is 6.5%. KRAS amplification is typically mutually exclusive from KRAS mutations and KRAS amplification is reported at an incidence of 10-15% in gastric cancer (GC) [1].
• The prognostic and/or therapeutic implications of KRAS amplification are not known in GEC, but were associated with acquired resistance to EGFR inhibitors and may also be responsible for primary resistance to anti-EGFR therapy in colorectal cancer (CRC) [2].
• NantOmics multiplexed selected reaction monitoring mass spectrometry (SRM-MS) analysis objectively quantifies multiple proteins from two formalin-fixed, paraffin-embedded (FFPE) tissue sections.
• By targeted proteomics, expression levels of multiple proteins including KRAS were determined in tumor biopsies from a retrospective cohort of GEC patients. We then compared SRM KRAS with KRAS FISH analysis.


Methods

Figure 1: Microdissected tumor areas from FFPE tissue blocks (n=418) of cell lines (n=25) and patients (n=933) were subjected to Liquid Tissue® digestion and MS to quantify KRAS protein levels in each patient sample. MS data was compared with FISH and HC analyses.

Figure 2: 25 GEC cell lines were assessed for KRAS protein expression by SRM-MS. A wide range of KRAS expression was observed (430-251,316 amol/µg). Red are FISH KRAS amplified; blue non-amplified.

KRAS expression in GEC Cell Lines

Figure 3: (A) Total KRAS protein quantification (amol/µg) across 393 individual tumors (some coupled with metastatic lesions) using SRM-MS. KRAS protein expression level = 0 was measured in 142 analyzed samples (not graphed). FISH performed when available (red, amplified; dark blue not amplified) (B, C) Correlation between FISH ratio (KRAS/CEP12) and KRAS protein expression level (amol/µg) in 88 samples with both FISH and SRM data was performed. Using Youden’s J statistic analysis, a cutoff of 1662 amol/µg was determined to optimize sensitivity/specificity. TPR- True Positive Rate; FPR- False Positive Rate.

FFPE Tissue: SRM-MS, FISH and NGS analyses; clinical & pathologic correlation

Figure 4: (A) The 1662 amol/µg cutoff was used to differentiate between high protein expression of KRAS and ‘normal’ KRAS. There was a trend towards worse overall survival (OS) associated with high KRAS protein expression (p value = 0.11) in univariate analysis. (B) The trend to worse survival was less pronounced with KRAS/CEP12 FISH ratio and/or NGS amplified samples (p value = 0.77).

Figure 5: Mice bearing MKN1 tumors were compared with mice bearing MKN1 KRAS-CRISPR tumors.

Combination of anti-MEK with anti-PI3K/mTOR

Conclusions

• Quantitative proteomic analysis in GEC patients was performed by SRM-MS. KRAS protein expression levels correlated with FISH KRAS/CEP12 ratio in GEC cell lines and tissues. SRM-MS KRAS protein expression ≥1662 amol/µg corresponded to KRAS gene amplification by FISH analysis. High KRAS protein expression was associated with worse OS, but with small numbers in this group (n=27) larger cohorts would be required to detect if this is statistically significant.

• KRAS gene knockdown using CRISPR technology led to inhibition of in vivo tumor growth in KRAS gene-amplified cell lines, and relatively less inhibition in non-amplified lines (data not shown), consistent with an oncogenic driver event.

• Combined inhibition of MEK and PIK3CA/mTOR (XL765) resulted in significant slowing of MNK-1 growth in culture. Murine models are ongoing.

• Novel lipid nanoparticle delivery systems for Dicer substrate short-interfering RNA (siRNA) in early phase clinical trials may be a promising approach to targeted inhibition of KRAS-amplified tumors.

• Patients are not tested for KRAS amplification as part of the routine diagnostic evaluation. Targeted multiplexed mass spec-based proteomics allows to test for KRAS amplification and consequent overexpression at the protein level, along with other targetable protein markers, and hence derive actionable intelligence that the physicians can potentially use for the management of oncologic malignancies.