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Investigation of KRAS Mutant Effect on Cell Signalling and Protein Expression Profiles in Microsatellite Stable Colorectal Cancer

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Hypothesis

Not all KRAS mutants are created equal and may cause different biological effects within tumour tissues that lead to different survival outcomes among patients.

Introduction

CRC is the 3rd most common cancer in Europe. Due to lack of symptoms CRC is very often diagnosed at a late stage in the disease, with a 5-year survival rate of <10%.

Microsatellite stable (MSS) CRC is the most common subtype of CRC with KRAS being the predominant mutation type in MSS CRC

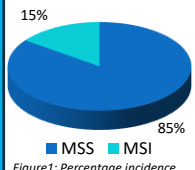


Figure1: Percentage incidence MSS CRC in CRC patients

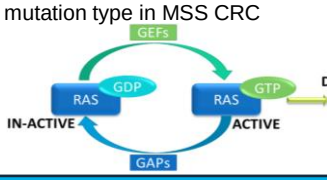
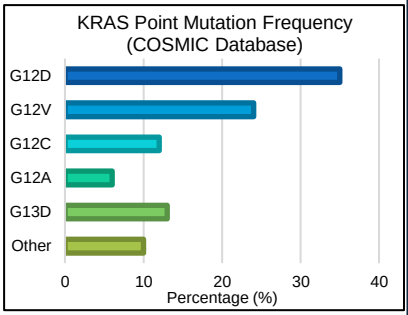


Figure2: RAS cycles between RAS-GDP (Inactive) and RAS-GTP (Active) states which lead to further interactions with downstream effectors of RAS producing different biological processes such as cell survival, cell migration and transcription

KRAS mutations can occur at codons 12, 13, 61, 63 & 146

- 80% CRC patients codon 12 mutation
- 18% CRC patients codon 13 mutation
- <5% KRAS mutations in codons 61, 63 & 146

Figure3: Percentage of KRAS point mutation frequency incidence of KRAS mutations occurring at codons 12 and 13 in CRC patients, according to the COSMIC Database.



KRAS WT and Mutant *in-vitro* Interactome

Different KRAS mutants have different interaction affinities with RAS effector proteins

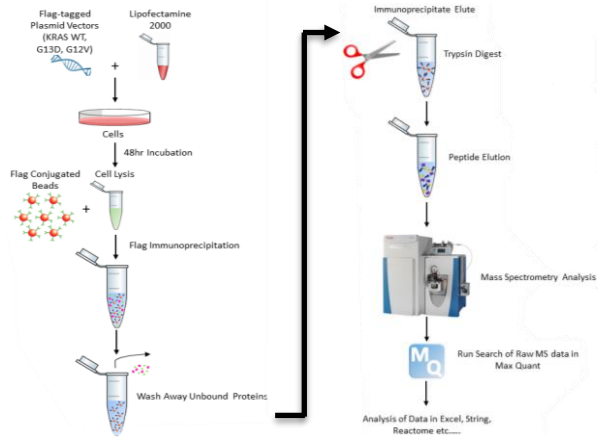


Figure4: Affinity purification protocol scheme to investigate KRAS WT, G13D & G12V interactome in-vitro, through high-put proteomics screening

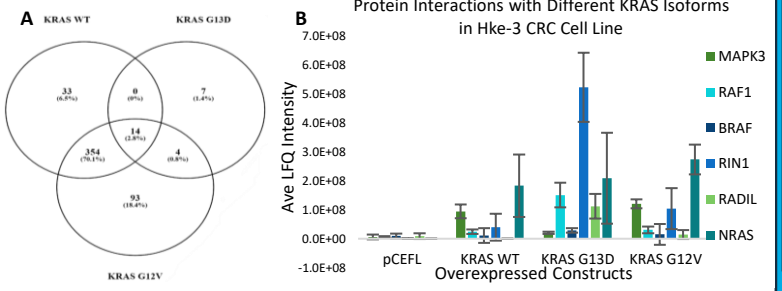


Figure5: (A) Venn Diagram showing the number of proteins that showed either a specific affinity, a shared affinity between two KRAS isoforms or proteins that had a common interaction with all three KRAS isoforms (WT, G13D & G12V). (B) Bar chart showing a number of proteins from the proteomics screening showing different interaction affinities between KRAS isoforms.

RADIL has a higher interaction affinity with KRAS G13D mutant compared to G12V

RIN1 interaction with KRAS mutants is cancer specific

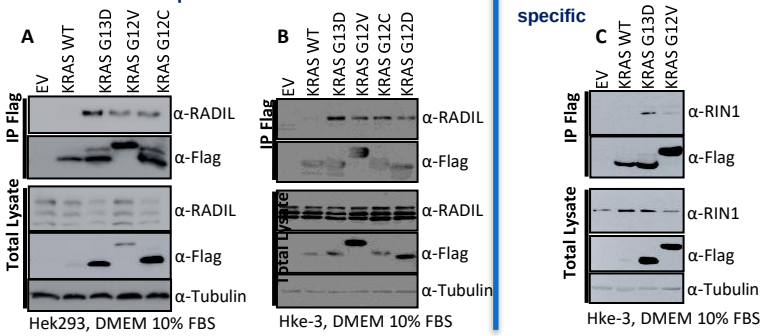


Figure6:Immunoprecipitation of proteins with FLAG-tagged plasmids transfected in Hek293 and Hke-3 cells. RADIL interaction shown with KRAS mutants G13D, G12V in (A) Hek293 and (B) Hke-3 CRC cell lines. (C) RIN1 interaction with KRAS G13D & G12V mutants in Hke-3 cells.

Proteomic Profiling of MSS CRC Patients

Patients with the same molecular subtype of CRC can cluster into different groups based on their individual proteomic expression profiles

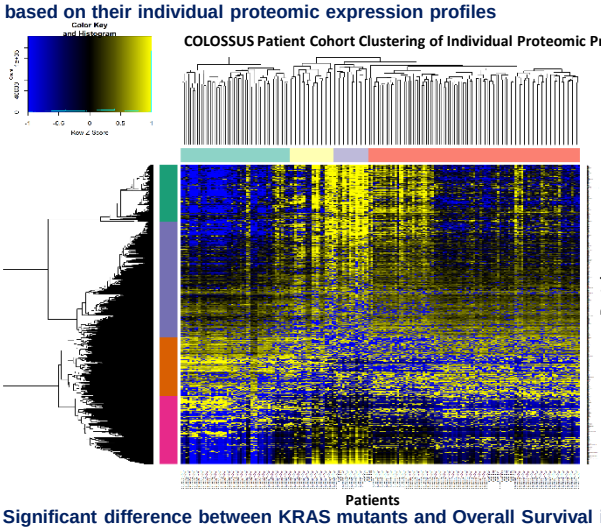


Figure7: Unsupervised Hierarchical clustering of the COLOSSUS cohort (MSS RAS mutant CRC patients) based on individual protein expression profiles, (x-axis = patients, y-axis = proteins)

Significant difference between KRAS mutants and Overall Survival in MSS CRC patient cohort

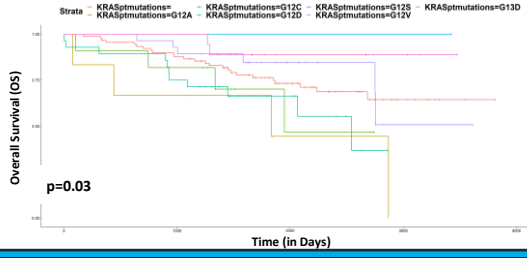


Figure8: Survival analysis of COLOSSUS cohort based on specific KRAS point mutation in patient tumour tissue (MSS RAS mutant CRC patients)

Conclusions

- Different KRAS mutants have differential interaction affinities with RAS effector proteins, leading to diverse interactomes between KRAS WT and mutants G13D & G12V
- RIN1 interaction with KRAS mutants is cancer specific
- Both RADIL and RIN1 (linked to migration and cell adhesion) have a higher interaction affinity for KRAS mutant G13D compared to G12V
- Patients of a similar molecular subtype of CRC (MSS KRAS mutant COLOSSUS patient cohort) can be further subdivided into clusters based on their individual proteomic profiles
- Different KRAS mutants are associated with a significant difference in overall survival of MSS KRAS mutant CRC patients

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