Ras-ERK and PI3k-mTOR Pathway Profiling in Solid Tumors and Implications for Clinical Oncology

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Abstract

Introduction: Ras-ERK and PI3K-mTOR pathways are chief regulators of cell proliferation, differentiation, survival, migration and metabolism. Alterations of these pathways are commonly seen in cancer pathogenesis. As next generation sequencing (NGS) platforms become more accessible to healthcare, the use of highly multiplexed mutational analysis for personalized medicine is on the rise. The ability to profile multiple signaling pathways can provide basis for targeted single agent or combinatorial cancer therapy.

Methods: Components of Ras-ERK pathway: KRAS, NRAS, HRAS and BRAF, and components of PI3K-mTOR pathway: PIK3CA, PTEN, AKT1 and STK11 were tested by next generation sequencing using the Trueseq Amplicon Cancer Panel on Illumina's Miseq. Formalin-fixed paraffin-embedded tissue sections from 5969 patients were subjected to DNA extraction and NGS. Immunohistochemistry using anti-PTEN clone 6H2.1 (DAKO) was used to analyze protein expression.

Results: Among 5969 cancer samples, a significant bias towards mTOR pathway was observed for breast carcinoma (42.7% cases mutated in mTOR pathway vs 0.4% cases mutated in ERK pathway), endometrial cancer (39.5% mTOR vs 3.4% ERK), ovarian surface epithelial carcinoma (17.5% mTOR vs 6.8% ERK), which may explain the success of mTOR inhibitors in these female prevalent/restricted cancers. Significant bias towards ERK pathway was observed for melanoma (6.7% mTOR vs 38.0% ERK) and pancreatic adenocarcinoma (2.9% mTOR vs 46.4% ERK). Colorectal adenocarcinoma and pancreatic adenocarcinoma were more likely to have alterations in both ERK and mTOR pathways compared with other tumor types. When NGS data was used instead of IHC for PTEN analysis, there were significantly fewer cases with PTEN alterations, highlighting the differences of the two techniques.

Conclusions: Pathway profiling reveals mTOR bias in female prevalent/restricted tumors and ERK bias in melanoma and pancreatic adenocarcinoma. Colorectal adenocarcinoma and pancreatic adenocarcinoma have tendency to have mutations in genes of both mTOR and ERK pathways, suggesting dual mTOR and ERK inhibitor therapy might be effective in these tumor types. Success of mTOR inhibitors in breast and endometrial cancers may also be a result of the low rate of ERK pathway activation.

**This abstract contains updated information since original submission.*

Methods

Formalin-fixed paraffin-embedded (FFPE) tissue sections were reviewed by pathologists to determine tumor%. For NGS, at least 10 unstained 5um FFPE slides, with tissue area ≥ 100 mm2, and tumor% ≥ 20% are required, optimal DNA amount is at least 250 ng. 45 genes of the Illumina Trueseq Cancer Panel, except for CDKN2A, SRC, FGFR3, were sequenced using Miseq. Data was analyzed by Miseq Reporter and in-house bioinformatics pipeline. Variants were manually checked and annotated, and were classified into 5 categories: pathogenic, presumed pathogenic, variant of unknown significance (VUS), presumed benign and benign. Pathogenic and presumed pathogenic variants were regarded as "mutated", benign and presumed benign variants were analyzed as "wildtype", VUS and indeterminate results were classified as "others". PTEN IHC was performed using anti-PTEN clone 6H2.1 (DAKO). Positive or "wildtype" was defined as staining intensity 0 and above and less than 50% tumor cells stained, negative or "mutated" was defined as staining intensity 1 and above and great than 50% tumor cells stained. Fisher exact test and bonferroni correction were used to assess statistical significance.

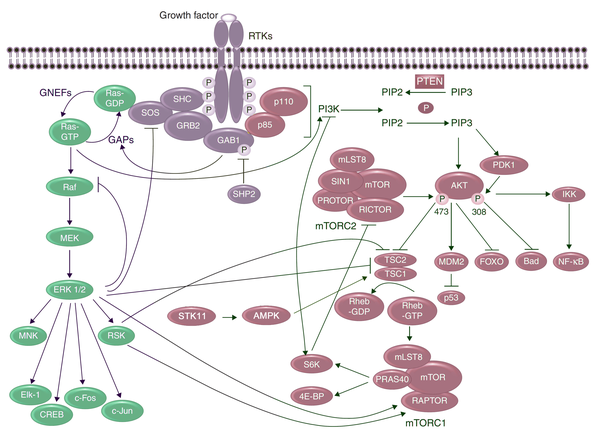

Figure 1 – ERK and mTOR pathways. Modified from reference 1.

Lineage	ERK Mutated	mTOR Mutated	Both Mutated	Both Wild Type	Others	Statistical Significance
Breast Carcinoma	3 (0.4%)	302 (42.7%)	13 (1.8%)	225 (31.8%)	165 (23.3%)	*
Ovarian Surface Epithelial Carcinomas	79 (6.8%)	204 (17.5%)	40 (3.4%)	620 (53.1%)	224 (19.2%)	*
Endometrium	13 (3.4%)	152 (39.5%)	57 (14.8%)	81 (21%)	82 (21.3%)	*
Melanoma	85 (38%)	15 (6.7%)	48 (21.4%)	54 (24.1%)	22 (9.8%)	*
Pancreatic Adenocarcinoma	111 (46.4%)	7 (2.9%)	76 (31.8%)	16 (6.7%)	29 (12.1%)	*
Colorectal Adenocarcinoma	118 (20.2%)	98 (16.8%)	211 (36.1%)	95 (16.3%)	62 (10.6%)	
Cholangiocarcinoma	7 (11.1%)	14 (22.2%)	4 (6.4%)	25 (39.7%)	13 (20.6%)	
Esophageal and Esophagogastric Junction Carcinoma		1 (16.7%)		2 (33.3%)	3 (50%)	
Extrahepatic Bile Duct Adenocarcinoma	1 (14.3%)	1 (14.3%)	1 (14.3%)	3 (42.9%)	1 (14.3%)	
Gastric Adenocarcinoma	3 (4.4%)	13 (19.1%)	5 (7.4%)	32 (47.1%)	15 (22.1%)	
Gastroesophageal Adenocarcinoma	3 (3.7%)	20 (24.4%)	2 (2.4%)	37 (45.1%)	20 (24.4%)	
Gastrointestinal Stromal Tumors (GIST)		2 (6.7%)		20 (66.7%)	8 (26.7%)	
Glioblastoma	9 (5.2%)	12 (7%)	1 (0.6%)	106 (61.6%)	44 (25.6%)	
Head and neck Squamous Carcinoma	1 (1%)	26 (26.8%)	2 (2.1%)	42 (43.3%)	26 (26.8%)	
Liver Hepatocellular Carcinoma		16 (45.7%)	1 (2.9%)	13 (37.1%)	5 (14.3%)	
Low Grade Glioma	1 (7.1%)	1 (7.1%)		10 (71.4%)	2 (14.3%)	
Lung Bronchioloalveolar carcinoma (BAC)			1 (50%)		1 (50%)	
Lung Non-small cell lung cancer (NSCLC)	110 (18.9%)	95 (16.3%)	61 (10.5%)	222 (38.1%)	95 (16.3%)	
Lung Small Cell Cancer (SCLC)	3 (7%)	10 (23.3%)		21 (48.8%)	9 (20.9%)	
Lymphoma	1 (8.3%)	4 (33.3%)		6 (50%)	1 (8.3%)	
Male Genital Tract Malignancy		2 (33.3%)		3 (50%)	1 (16.7%)	
Malignant Solitary Fibrous Tumor of the Pleura (MSFT)				1 (100%)		
Multiple Myeloma			1 (100%)			
Neuroendocrine tumors	8 (4.9%)	14 (8.6%)	2 (1.2%)	109 (66.9%)	30 (18.4%)	
Nodal Diffuse Large B-Cell Lymphoma	1 (33.3%)	1 (33.3%)		1 (33.3%)		
Non Epithelial Ovarian Cancer (non-EOC)	1 (2%)	16 (32%)		25 (50%)	8 (16%)	
Pituitary carcinomas, Oligodendroglioma				2 (100%)		
Prostatic Adenocarcinoma		18 (36.7%)	2 (4.1%)	20 (40.8%)	9 (18.4%)	
Retroperitoneal or Peritoneal Carcinoma					1 (100%)	
Retroperitoneal or Peritoneal Sarcoma		2 (9.1%)		15 (68.2%)	5 (22.7%)	
Small Intestinal Malignancies	15 (35.7%)	6 (14.3%)	7 (16.7%)	10 (23.8%)	4 (9.5%)	
Soft Tissue Tumors	7 (4.1%)	21 (12.1%)		100 (57.8%)	45 (26%)	
Thymic Carcinoma	1 (9.1%)			7 (63.6%)	3 (27.3%)	
Thyroid Carcinoma	11 (44%)	3 (12%)	2 (8%)	5 (20%)	4 (16%)	
Uveal Melanoma				6 (54.6%)	5 (45.5%)	

Table 1 – ERK and mTOR pathway profiling in different cancer lineages. Statistically significant lineages are marked by "*".

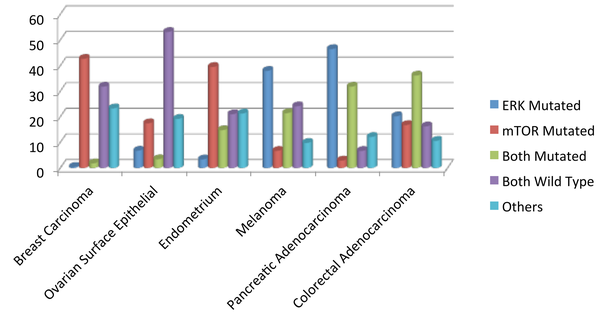

Figure 2 – ERK and/or mTOR pathway bias in different cancer lineages.

Results

When IHC data was used for PTEN and NGS data was used for the other 7 genes for analysis, significant bias towards mTOR pathway was observed for female prevalent/restricted tumors: breast carcinoma (42.7% cases mutated in mTOR pathway vs 0.4% cases mutated in ERK pathway), endometrial cancer (39.5% mTOR vs 3.4% ERK), ovarian surface epithelial carcinoma (17.5% mTOR vs 6.8% ERK (Table 1, figure 2), which may explain the success of mTOR inhibitors in these female prevalent/restricted cancers. Significant bias towards ERK pathway was observed for melanoma (6.7% mTOR vs 38.0% ERK) and pancreatic adenocarcinoma (2.9% mTOR vs 46.4% ERK). Colorectal adenocarcinoma (36.1% both mutated) and pancreatic adenocarcinoma (31.8% both mutated) were more likely to have alterations in both ERK and mTOR pathways compared with other tumor types. When NGS data was used for PTEN analysis, there were significantly fewer cases with PTEN alterations, which might suggest loss of PTEN protein is also due to abnormalities other than sequence changes, such as epigenetic changes, post-transcriptional regulation and PTEN protein stability regulation, etc. For details, please see another poster from Caris (ST62, A. Ghazalpour).

Conclusions

- Pathway profiling reveals mTOR bias in female prevalent/restricted tumors and ERK bias in melanoma and pancreatic adenocarcinoma.
- Colorectal adenocarcinoma and pancreatic adenocarcinoma are likely to have mutations in genes of both mTOR and ERK pathways, suggesting dual mTOR and ERK inhibitor therapy might be effective in these tumor types.
- Success of mTOR inhibitors in breast and endometrial cancers may also be a result of the low rate of ERK pathway activation.

References

De Luca et al. *The RAS/RAF/MEK/ERK and the PI3K/AKT signaling pathways: role in cancer pathogenesis and implications for therapeutic approaches.* Expert Opin. Ther. Targets (2012) 16(Suppl.2):s17-s27.