

Characterization of Signaling Pathways in Solid Tumors Using Comprehensive Genomic Profiling

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INTROD UCTION

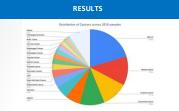
Malignant tumor formation is driven by aberrations in signaling pathways controlling cellular growth, proliferation and death. A large next-generation sequencing (NGS) panel which indudes not just the well known genes in these pathways but also the lesser studied members offers a more comprehensive assessment of the involvement of these pathways. We thus aimed to profile the samples analyzed at our facility over the last 5 years to characterize their cancer signaling pathways.

AIMS

- To profile samples analyzed on a large nextgeneration sequencing (NGS) panel evaluating SNVs, indels, CNVs and fusions.
- To map the altered genes to frequently altered cancer signaling pathways
- To characterize the cancer signaling pathways affecting specific cancers

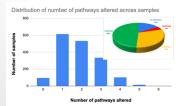
METHODS

A set of 2015 formalin-fixed, paraffin embedded (FFPE) samples across multiple cancers was profiled using the Illumina TSO500 panel. Variants (SNVs and indels), amplifications and fusions were classified according to the Association for Molecular Pathology (AMP) tiering system. Tier 1 and 2 variants were taken forward and mapped into 11 different literature curated signaling pathways. Mutation profiles were analyzed by cancer type and comutation rates of genes in the same pathway were also calculated.



Distribution of cancers in the cohort of 2015 patients. Cancers predominantly represented in the cohort were lung (20.4%), breast (12.4%), ovarian (11.1%) and colon (10.5%) cancers with other cancers represented in smaller numbers (N< 200).

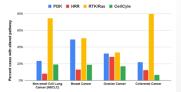
Average number of alterations calculated for top 4 observed cancers. Alterations (SNV, indels, CNV, fusions) were observed in multiple genes across different cancer types. The average number of alterations per case was 3.7 in colon, 3.1 in breast, 3.0 in lung and 2.6 in ovarian cancer.



Pathways activated in each sample. The altered genes were mapped to different signaling pathways that are frequently altered in cancer. Almost half the samples had more than 1 pathway altered. The average number of pathways altered per case was 1.87. The main contributors were KRAS in colon, EGFR in lung and ERBB2 and FGFR1 in breast cancers.

PDK		RTKRas		Cell Cycle	HRR
AKT1	AKT1	PTEN	FOF9	CONNOA	ATM
PROCA	ALK	PTPNII	FGFR4	891	BRCAT
PTEN	APC	RET	LRP18	ABRIXING1	BRCAJ
STK11	AR	R061	MAP2K2	8701	MLH1
AKT2	BRAF	ACVR1	INAP2K4	OCN01	ATR
MTOR	EGFR	AKT2	MAPSK1	OCN03	8/021
P9K3C25	ER682	ANAT	MAPK1	OCNE1	CDKNG
PROCE	FOF#1	EPHA3	NRG1	CDK12	CHEKT
PRORI	PGPR2	EPHAS	NTEK2	CDKA	OHDO
PH3R2	PGPR3	EP003	POGFRB	COHE	FANCE
PPP2R1A	GNAS	ERREN	PPN/IO	CDKN/B	MRE11
RICTOR	HRAS	FOF1	PRIORISA	PLK2	NIN
RP50x81	KDR	FOF10	PTOH1	PPM10	PAL82
T9C1	KIT	FGF19	PTPRD	RA050	RADS18
TBC2	KRAS	FGF2	PTPRS		RADS10
	MAP2K1	FGF3	PTPRT		RADS10
	MET	FGF4	RAF1		RA0540
	NPT	FOFS	RASAL		
	NRAS	FOF6	RHOA		
	NTEX1	FGF7	871		
	POGERA	FGER	580		

List of altered genes mapped to the top 4 most frequently altered pathways. The genes in bold are those which have well established clinical significance and are found on smaller panels.



Pathways activated across different cancer types. The top 4 most frequently altered pathways were receptor tyrosine kinase (RTK) / Ras, phosphatidylinositol-3 kinase (PI3K), homologous recombination repair (HRR) and cell cyde (CC). RTK/Ras was altered in 71.6% colon, 74.5% lung and 50.6% breast cancers. PI3K pathway was altered most in breast (49%) and ovarian (32.3%) cancers. RTK/Ras and PI3K pathways were equally affected in breast (RTK/Ras-50.6%, PI3K-49%) and ovarian (RTK/Ras-33.6%; PI3K-32.3%) cancers. The CC pathway was altered at similar levels in lung (19%), breast (18.9%) and ovarian (17%) cancers compared to 6.6% in colon cancer. The HRR pathway was altered more in owarian (28.3%) cancer compared to breast (12.9%), colon (12.3%) or lung (8%) cancers.

RESULT

Cancer Type	RTK/Ras	PI3K	HRR	CC	
Breast Cancer	18.5	17.3	0.4	2.4	
Colorectal Cancer	30.3	7.1	2.8	1.4	
Non-small Cell Lung Cancer (NSCLC)	32.8	14.6	0.7	3.2	
Ovarian Cancer	12.1	10.8	1.8	1.3	

Multiple genes are altered in the same pathway. Rates of co-mutation were calculated as the percent of cases which had alterations in 2 or more genes in the pathway. HRR and CC pathways showed low rates of co-mutation (~< 3.5%). In contrast, RTK/Ras (12.1%-32.8%) and PI3K (7.1%-17.3%) pathways showed higher co-mutation rates across all 4 cancer types. In NSCLC, the RTK/Ras pathway was altered mainly in EGFR, KRAS, ERBB2, MET and ALK with EGFR alterations observed with highest frequency. The most frequent RTK/Ras pathway co-mutated gene with EGFR was MET. In colon cancer, the RTK/Ras pathway was altered predominantly in KRAS with alterations observed to a lesser extent in BRAF. PTEN. MET and EGFR. APC was the most frequently co-mutated with KRAS. PI3K pathway alterations were largely observed in PIK3CA in breast cancer. In ovarian cancer. the main gene altered in the PI3K pathway was found to be PIK3CA with PTEN. PIK3R1 and PIK3CB altered to a lesser extent.

CONCLUSIONS

Growth and proliferation pathways are altered more in certain cancers than others, however, the CC pathway is altered to a lesser extent but across cancers. The HRR pathway is predominantly altered in ovarian and prostate cancers. RTK/Ras and PI3K pathways have been well studied with smaller panels and established genes; however, large panels allow for indusion of additional components of these pathways, and assessment of HRR and CC pathways more effectively.