IRCCS-GC: copy number alteration - Illumina HiSeq 2500, Targeted capture

Molecular Methods Description:

DNA extracted from PDX models along with a sample of normal germline DNA from each patient were utilized for targeted next generation sequencing of a bait set of 243 genes selected based upon their alteration in prior studies of gastroesophageal cancer (the “243-GEA gene panel” was designed using SureSelect DNA platform; Agilent genomics). Additionally, hybrid capture using the human bait set followed by sequencing was performed on a NOD-SCID mouse germline DNA sample.

NGS was performed on an Illumina HiSeq2500 sequencer.

Analysis Description:

250-bp read pairs were aligned to the hg19 reference sequence using the BWA and data were sorted and duplicate-marked using Picard tools. The alignments were further refined using the Genome Analysis Toolkit (GATK) for localized realignment around indel sites . Recalibration of quality scores was also performed using the GATK.

Somatic events were identified with MuTect v1.1.4 and annotated using Variant Effect Predictor v79 (VEP). We used the SomaticIndelDetector tool that is part of the GATK for indel calling. Variants detected in PDXs that were also identified in murine DNA were filtered and removed. Only non-synonymous mutations were considered (missense, nonsense, splice donor, splice acceptor, start/stop lost, frameshift mutations, in-frame insertions/deletions).

RobustCNV was used for the identification of copy number variants. Read depth at informative capture targets in tumor samples was calibrated to estimate the copy ratio using depths observed in a panel of normal (non-cancer) diploid genomes. The copy-ratio profiles were then segmented using the circular binary segmentation (CBS) algorithm. Segments were assigned gain, loss, or normal-copy calls using a cutoff derived from the within-segment standard deviation of post-normalized mapping depths and a tuning parameter which was set based on comparisons to array-CGH calls in separate validation experiments.

Table:

243-GEA GENE PANEL				
AKT1	AKT2	AKT3	ALK	
ALPK2	APC	AR	ARAF	ARHGAP26
ARHGAP6	ARID1A	ARID1B	ARID2	ATM
AXL	B2M	BAK1	BCL2	BCL2A1
BCL2L1	BCL2L12	BCL2L2	BCL6	BCL9
BCOR	BCORL1	BIRC5	BLM	BRAF
BRCA1	BRCA2	BRD4	CA9	CACNA2D3
CASP3	CASZ1	CCND1	CCND2	CCND3
CCNE1	CD274	CD44	CDH1	CDK1

CDK12	CDK2	CDK4	CDK5	CDK6
CDK8	CDK9	CDKN1A	CDKN1B	CDKN1C
CDKN2A	CDKN2B	CDKN2C	CHL1	CHRD
CIC	CLDN18	CRIP1	CRKL	CSMD1
CTNNA1	CTNNA3	CTNNB1	CTSB	CXCL10
DOCK2	E2F1	E2F3	EGFR	ELF3
ELMO1	EP300	EPHB3	EPHB6	ERBB2
ERBB2IP	ERBB3	ERBB4	ETV4	EYA4
FAM190A	FAT1	FAT2	FAT3	FAT4
FBXW7	FGF19	FGF3	FGF4	FGFR1
FGFR2	FGFR3	FGFR4	FHIT	FRS2
GAB1	GAB2	GATA4	GATA6	GLI3
GLIPR1	GNAS	HES1	HGF	HLA-A
HLA-B	ID1	IDH1	IDH2	IGF1R
IGF2	IGF2BP3	IGF2R	IL8	ING5
IRF2	JAK2	KDM6A	KEAP1	KIF13A
KIT	KLF12	KLF5	KMT2D	KRAS
LARP4B	LRP1B	LRP6	MACF1	MACROD2
MAGI3	MAP2K1	MAP2K2	MAP2K2	MAP2K4
MAP3K1	MAP3K5	MAP3K7	MAP3K8	MAP3K9
MAPK1	MAPK3	MCL1	MDM2	MECOM
MET	MLL3	MSH2	MSH6	MTOR
MUTYH	MYB	MYC	MYD88	NEGR1
NEIL2	NF1	NFE2L2	NKX2-1	NOTCH1
NOTCH2	NOTCH3	NOTCH4	NR2F2	NRAS
PAK1	PALB2	PARD3B	PARK2	PAX9
PBRM1	PCGF6	PDCD1LG2	PDE4D	PDGFRA
PDGFRB	PGM5	PHLDA1	PIK3CA	PIK3CG
PIK3R1	PLK2	POLB	POU5F1B	PREX2
PRKCi	PTCH1	PTCH2	PTEN	PTPN23
PTPRD	RASA1	RASSF7	RB1	RBPJ
RhoA	RICTOR	RNF43	RPTOR	RUNX1
RUNX1T1	RUNX3	SFRP4	SMAD2	SMAD3
SMAD4	SMARCA4	SMARCA5	SMO	SMURF1
SOHLH2	SOX2	SOX9	SRC	STAT3
STAT6	STK11	SYNE1	TCF7L1	TCF7L2
TERC	TERT	TGFBR2	TLN1	TLR4
TP53	TP63	TP73	TRAF2	TSC1

TSC2	USP9X	VEGFA	VPS13A	WT1
WWOX	YAP1	ZNF217	ZNF750	