Variant calling and fusion gene detection in formalin-fixed paraffin-embedded tumor tissue using RNA capture sequencing

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aim

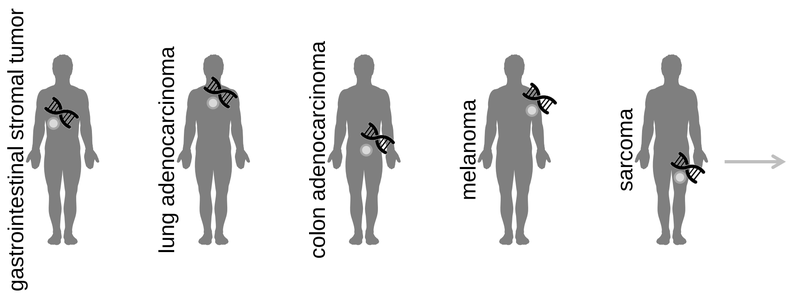
identification of genetic aberrations in formalin-fixed paraffin-embedded (FFPE) tissue of solid tumors using mRNA capture sequencing

study design

validation of analysis pipeline

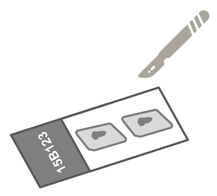
cohort I (n = 16)

different cancer types with a known aberration



FFPE RNA isolation

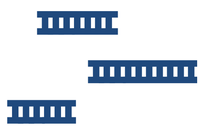
macrodissection and miRNeasy FFPE Kit



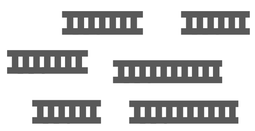
TruSight RNA Pan-Cancer Panel sequencing

1st strand cDNA synthesis

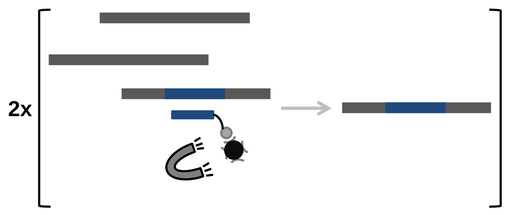
2nd strand cDNA synthesis and 3' end adenylation



adapter ligation and 1st PCR amplification

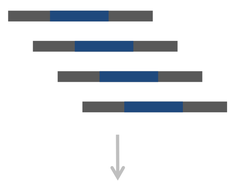


probe hybridization and capture of stranded RNA sequencing libraries



targeting 1385 cancer genes

captured library clean-up and 2nd PCR amplification



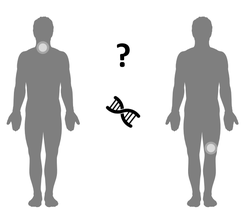
sequencing (2 x 75) on MiSeq

FastQC
Trimmomatic
STAR
Picard Tools
FusionCatcher - VarScan

identification of new genetic aberrations

cohort II (n = 17)

FISH fusion gene-negative alveolar rhabdomyosarcoma (ARMS)



FISH fusion gene-negative undifferentiated round cell sarcoma (URCS)

TruSeq RNA Access sequencing

targeting 21,000 human mRNA genes - NextSeq 500 (2 x 75)

results

cohort I - confirmation of genetic aberrations identified during the diagnostic workup of solid tumors

cohort II - identification of fusion transcripts in FISH fusion gene-negative sarcomas

1. identification of a pathognomonic fusion transcript in 6/17 patients

ARMS

patient	fusion transcript	SP
P18	PAX3-FOXO1	11
P25	PAX3-FOXO1	13

SP: spanning pairs, i.e. the number of paired-end reads supporting the fusion.

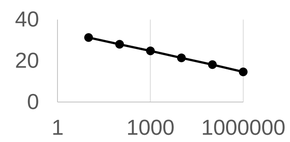
patient	fusion transcript	SP
P26	EWSR1-ERG	3
P27	EWSR1-NFATC2	19
P28	EWSR1-NFATC2	19
P29	EWSR1-FLI1	4

URCS

2. qPCR validation of novel fusion transcripts in remaining 11 patients

primerXL assay design for 20 novel fusion transcripts

60-mer synthetic templates for assay validation



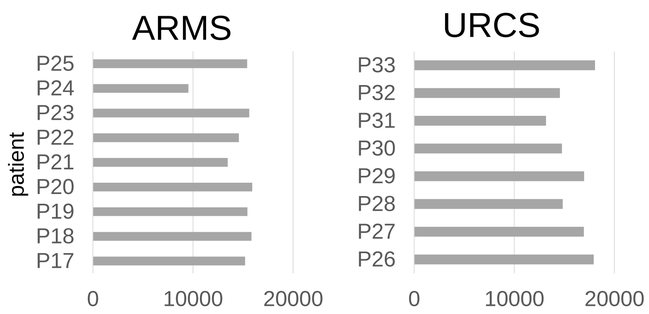
E = 1.997 ± 0.019
R^2 = 0.998
slope = -3.328 ± 0.045
Y-intercept = 34.712 ± 0.176

9/20 fusion transcripts were validated on FFPE tumor material

COPS3-TOM1L2, NCOA1-DTNB, WWTR1-LINC01986, IGK@-BAGE2, PITPNC1-CACNG4, PLAA-MOB3B, PTPRG-PPP4R2, BRD4-LEUTX and AP1B1-CHEK2

cohort II - identification of sequence variants in FISH fusion gene-negative sarcomas

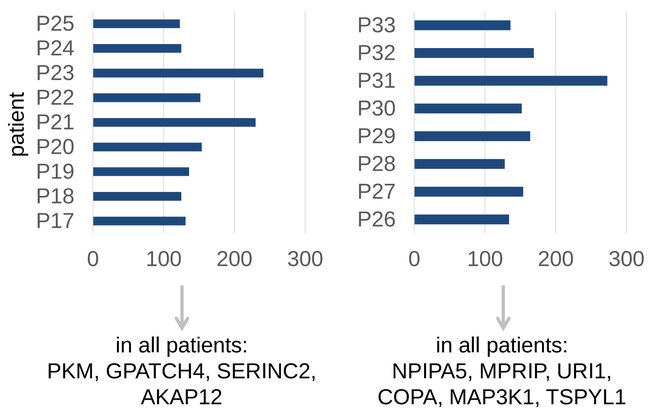
total variant numbers
range: 9545 - 18,067
median: 15,413



variant filtering

gnomAD allele frequency
< 0.01%
Sequence Ontology term
impact high or moderate
RNA editing
removed (except confirmed somatic variants (COSMIC))

filtered variant numbers
range: 123 - 273
median: 152



recurrently detected variants

in all patients:
PKM, GPATCH4, SERINC2, AKAP12

in all patients:
NPIPA5, MPRIP, URI1, COPA, MAP3K1, TSPYL1

conclusion

In this study, we successfully applied mRNA capture sequencing to identify fusion transcripts and sequence variants in FFPE tissue of solid tumors, without making use of matching normal RNA. We demonstrated that RNA capture sequencing may enhance the detection of pathognomonic fusion genes in sarcoma. Additionally, recurrent sequence variants were identified in fusion gene-negative sarcomas. Our results may provide new insights into the underlying genetic causes of these malignancies.