Supplementary Material

Title

Specific Recognition of an *FGFR2* Fusion by Tumor Infiltrating Lymphocytes from a Patient with Metastatic Cholangiocarcinoma

Authors

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Supplementary Figure 1. (A) The heatmap depicts discordant read pairs (i.e., read pairs that mapped to two different partner genes, each on the opposite side of the fusion breakpoint), as reported by each fusion caller (rows) for all the tumor samples from four *FGFR2* fusion-positive patients (columns). **(B-D)** Results of the automated electrophoresis (left panels) and Sanger sequencing (right panels) of the amplification products generated by RT-PCR using fusion-specific primers for patient 2 (B), 3 (C) and 4 (D). cDNA from patient 3 was used as a negative control for patient 2; cDNA from patient 2 was used for patient 3; cDNA from patient 1 was used as negative control for patient 4. NT, no template. **(E)** Expression levels of each *FGFR2* fusion in individual tumors from patients 1-4. Each dot in the left panel represents a separate tumor; tumors from patients 2-4 were clustered together in the right panel for the purpose of statistical analysis (unpaired t-test; $p<0.0001$). RBPKM, read base per kilobase per million.

Supplementary Figure 2. (A) Schematic representation of chromosome 10. Coordinates corresponding to transcript breakpoint regions for each fusion partner from four patients are indicated, as is their orientation and the distance from the transcript breakpoint of *FGFR2*. (B) Schematic representation of genes encoding the FGFR2 fusions, as well as the wild type FGFR2 and the individual partner proteins. Exon composition of each wild type gene was imported from the NCBI Reference Sequence Database (RefSeq). Because the fusion reconstruction was performed based on whole transcriptome and not whole genome sequencing, it was not possible to distinguish whether the fusion breakpoints occurred at the exon/intron boundaries or within the neighboring introns of each participating gene. Note that exons 1 from both *TDRD1* and *CEP55* refer to 5' untranslated regions (UTR) of the corresponding genes, and do not contribute to canonical protein translation (which starts from ATG in exon 2). To display the read coverage for each exon, RNA sequencing data from representative tumors was aligned to the reference genome hg38 using the STAR aligner.

Supplementary Figure 3. Mutational landscape in FGFR2 fusion-positive and negative ICC cases. Statistical analysis was performed using the unpaired t-test. **(A)** Comparison of the total mutation number ($p=0.609$). **(B)** Comparison of the number of immunogenic mutations, as determined by previous TIL screening ($p=0.535$).

Supplementary Figure 4. (A) Representative sections of tumor C resected from patient 1, stained for surface MHC class I (upper panel) or class II molecules (lower panel). The staining patterns were consistent among all three tumors resected from patient 1. (B) Transcript expression levels (in transcripts per million, TPM) for all class I and II molecules detected in three tumors from patient 1.

Supplementary Figure 5. (A) Flow cytometric analysis of 4-1BB expression on $CD8^+$ TILs from the same experiment depicted in Figure 2B. (B) Results of multicytokine analysis on the supernatants from the experiment depicted in Figure 2C.

Supplementary Figure 6. TIL from patients 2, 3 and 4 did not recognize any of the patient-specific *FGFR2* fusions. (A) TILs from patient 2 (A), 3 (B) and 4 (C) were co-cultured with autologous DCs that were either pulsed with patient-specific FGFR2 fusion 26-mer peptides or were transfected by the corresponding minigenes. The following day, IFN-γ production was measured using IFN-γ ELISPOT (upper panels), while the 4-1BB expression on $CD8^+$ T cells (middle panels) and $CD4^+$ T cells (lower panels) was assessed using flow cytometry. A tandem minigene (TMG) encoding point mutations from another patient (Irrel. TMG) and DMSO were used as negative controls for minigene and peptide testing, respectively. 25-mer peptides or TMGs encoding point mutations previously identified as neoantigens in TIL screens from the same patients (see Supplementary Table 2) were used as positive controls. PMA/ionomycin (PMA) was used as a non-specific positive control.

Supplementary Figure 7. (A) Transduction efficiencies for TCR1, as assessed by flow cytometry for mouse TCR-beta constant chain (mTCR-β) expression. Histograms depict mTCR-β staining on untransduced (shaded) and transduced (unshaded) cells from two healthy donors; numbers indicate the percentage of transduced cells. Graphs were gated on live $CD3^+$ lymphocytes. (B) All peptides that spanned the FGFR2-TDRD1 breakpoint (each represented with a single dot) and that were predicted to bind to any of the paired MHC-II molecules from patient 1' tumor using NetMHCIIpan-4.0 were ranked by their affinity (with highest affinity-binders on the top). The strongest binder to HLA-DRB1*04:04 that overlapped with the experimentally determined FGFR2-TDRD1 minimal epitope is indicated with an arrow. (C)

Flow cytometric analysis of HLA-DR expression on the surface of COS7 cells following co-transfection with plasmids encoding HLA-DRA1*01:01 and HLA-DRB1*04:04. (D) Automated electrophoresis for the FGFR2-TDRD1 amplification product from the cDNA of COS7 cells that were mock-transfected ("Mock") or were transfected with an expression plasmid encoding the fusion construct ("Fusion"). NT, no template. Primers for the wild type African green monkey *GAPDH* were used to generate a positive control.

Supplementary Table 1. Patient and tumor characteristics

Pt ID	Age / Sex	FGFR2 Fusion Identified	Initial AJCC Stage	Differentiation Status	Prior Therapies	Sites of Metastatic Disease at Presentation to the NIH	TIL Harvest Location	Outcome After ACT
1	48/F	FGFR2-TDRD1	IIB	Well	Gemcitabine+cisplatin, SBRT, infigratinib	Lung	Lung	N.T.
2	53/F	FGFR2-CEP55	IB	Moderate	Cisplatin+gemcitabine, gemcitabine, docetaxel	Lung, liver	Lung	PR
3	58/F	FGFR2-WAC	II	Moderate	Gemcitabine, futibatinib	Lung, liver, adrenal, LN	Lung	PD
4	51/F	FGFR2-WAC	IB	Moderate	Gemcitabine+oxaliplatin, HAI-Floxuridine, gemcitabine	Lung	Lung	PD
5	47/F	None	IV	Unknown	Gemcitabine+carboplatin	Lung, liver, bone	Liver	N.T.
6	47/F	None	IIIB	Moderate	Hyperthermic 5-flouorouracil+gemcitabine, gemcitabine+cisplatin,	Lung, liver	Liver	N.T.
7	60/M	None	IIIB	Moderate to poor	Gemcitabine+cisplatin x2, FOLFIRI, FOLFOX, gemcitabine	Liver, peritoneum, LN	Peritoneal	PD
8	45/F	None	IV	Moderate to poor	Gemcitabine+capecitabine, gemcitabine+carboplatin, capecitabine, nab-paclitaxel, dasatinib	Lung, LN	Lung	N.T.
9	49/M	None	IV	Moderate	Gemcitabine+cisplatin, cisplatin, IDH305 (IDH1 inhibitor), ivosidenib	Lung, liver, bone	Liver	N.T.
10	44/M	None	IV	Poor	TACEx3, gemcitabine+cisplatin	Lung, liver, LN	Lung	N.T.
11	67/F	None	IV	Poor	Gemcitabine+cisplatin	Liver, peritoneum, LN, bone	Peritoneal	SD
12	55/F	None	IV	Poor	Gemcitabine+cisplatin, cisplatin	Lung, liver, LN	Liver	N.T.

AJCC, American Joint Committee on Cancer; SBRT, stereotactic body radiation therapy; HAI-floxuridine, hepatic arterial infusion with floxuridine; FOLFIRI, folinic acid (leucovorin), 5-fluorouracil and irinotecan; FOLFOX, folinic acid (leucovorin), 5-fluorouracil and oxaliplatin; TACE, trans-arterial chemoembolization; LN, lymph node; TIL, tumor infiltrating lymphocyte; ACT, adoptive cell therapy; PD, progressive disease; PR, partial response; N.T., not treated

Supplementary Table 2. Results of TIL screening for recognition of cancer-specific point mutations

Pt ID	No of tumors sequenced	Mutations Identified (n)	Mutations screened (n)	Mutation (neoantigen) recognized by T cells	Neoantigen Variant Key	HLA Class Restriction	Reference
1	3	162	156	*RSU1*^{P150T}	10:16794952-16794952 G>T	II	Parkhurst, 2019
2	3	219	26	*ERBB2IP*^{E805G}	5:65349560-65349560 A>G	II	Tran, 2014
3	3	59	49	*TLK1*^{R170L}	2:171906620-171906620 C>A	II	
				MB21D2^{M69I}	3:192635423-192635423 C>A	II	
				PAPLN^{R75H}	14:73712397-73712397 G>A	II	
				DDX41^{K407E}	5:176939332-176939332 T>C	II	
4	1	59	35	*HIST1H2BE*^{E72V}	6:26184238-26184238 A>T	I	Parkhurst, 2019
5	1	73	64	None			
6	1	27	18	None			
7	3	84	56	*TUBGCP6*^{R611Q}	22:50664480-50664480 C>T	II	Parkhurst, 2019
				ECE2^{A40T}	3:183994347-183994347 G>A	II	
8	Multiple	136	N.S.				
9	2	108	N.S.				
10	2	51	35	None			
11	2	230	84	*TES*^{K62N}	7:115889146-115889146 A>T	I	Parkhurst, 2019
				HYDIN^{G4555V}	16:70867805-70867805 C>A	II	
				ACLY^{R794W}	17:40024980-40024980 T>A	II	
12	1	994	207	*NBAS*^{C144S}	2:15679429-15679429 C>G	I	Parkhurst, 2019
				EIF4A3^{D169Y}	17:78113807-78113807 C>A	II	

N.S., not screened

Supplementary Table 3. FGFR2 fusion sequences and genomic locations of the fusion partners

Pt	Fusion	5' Partner		3' Partner		Breakpoint-spanning amino acid sequence
		Transcript ID	Breakpoint coordinate (exon)	Transcript ID	Breakpoint coordinate (exon)	
1	*FGFR2-TDRD1*	NM_000141.5	10:121483698 (17)	NM_198795.2	10:114187826 (5' UTR*)	EDLDRILTLTTNE|ASMSVKSPFNVMS
2	*FGFR2-CEP55*	NM_000141.5	10:121483698 (17)	NM_018131.5	10:93500040 (5' UTR*)	EDLDRILTLTTNE|TISEMSSRSTKDL
3	*FGFR2-WAC*	NM_000141.5	10:121483698 (17)	NM_016628.5	10:28617657 (12)	EDLDRILTLTTNE|ASRLREEAHNMGT
4	*FGFR2-WAC*	NM_000141.5	10:121483698 (17)	NM_016628.5	10:28589736 (5)	EDLDRILTLTTNE|PYDSADDWSEHIS

*5' untranslated region (UTR) of each gene is annotated as exon 1 in the RefSeq database.

Supplementary Table 4. Primer sequences used in this study

Amplification product	Forward/reverse	Sequence (5'-3')	Product size (bp)
GAPDH	forward	ACC ACA GTC CAT GCC ATC AC	452
	reverse	TCC ACC ACC CTG TTG CTG TA	
FGFR2-WAC (Pt 4)	forward	ATT CCC GTG GAG GAA CTT TT	387
	reverse	TGT TGC TTG CAT CAC CTC TC	
FGFR2-WAC (Pt 3)	forward	ATT CCC GTG GAG GAA CTT TT	381
	reverse	CAC ACC ATG AAG GAA TTC TGA TT	
FGFR2-CEP55	forward	ATT CCC GTG GAG GAA CTT TT	399
	reverse	GCC TCA AGG ACT CGA ATT TT	
FGFR2-TDRD1	forward	TCC TTC GGG GTG TTA ATG TG	466
	reverse	GGT TTG CTC ACA AAG CAA AAA	
African green monkey GAPDH (for COS7 cells)	forward	CAG CCT CAA GAT CGT CAG CA	134
	reverse	TCT TCT GGG TGG CAG TGA TG	

Supplementary Table 5. Sequences corresponding to FGFR2 fusion and control minigenes

Patient	Fusion	Amino Acid Sequence	Length
1	FGFR2-TDRD1	MGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPY PGIPVEELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLT TNE|ASMSVKSPFNVMSRNNLEAPPCKMTEPFNFEKNENKLPPHESLRSPGTLPNHPNFRL KSSENGNKKNNFLLCEQTKQYLASQEDNSVSSNPNGINGEVVGSKGDRKKLPAGNSVSP PSAESNSPPKEVNIKPGNNVRPAKSKKLNKLVENSLSISNPGLFTSLGPPLRSTTCHRCGL	301
1	FGFR2	MLSDLVSEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYS YDINRVPEEQMTFKDLVSCTYQLARGMEYLASQKCIHRDLAARNVLVTENNVMKIADFGLA RDINNIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPYPGIPV EELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLTTNEEY LDLSQPLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYEPCLPQYPHINGSVKT	295
1	TDRD1	MASMSVKSPFNVMSRNNLEAPPCKMTEPFNFEKNENKLPPHESLRSPGTLPNHPNFRLKS SENGNKKNNFLLCEQTKQYLASQEDNSVSSNPNGINGEVVGSKGDRKKLPAGNSVSPPS AESNSPPKEVNIKPGNNVRPAKSKKLNKLVENSLSISNPGLFTSLGPPLRSTTCHRCGLFG SLRCSQCKQTYYCSTACQRRDWSAHSIVCRPVQPNFHKLENKSSIETKDVEVNNKSDCPL GVTKEIAIWAERIMFSDLRSLQLKKTMEIKGTVTEFKHPGDFYVQLYSSEVLEYMNQLSA	300
2	FGFR2-CEP55	MGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPY PGIPVEELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLT TNE|TISEMSSRSTKDLIKSKWGSKPSNSKSETTLEKLKGEIAHLKTSVDEITSGKGKLTDKE RHRLLEKIRVLEAEKEKNAYQLTEKDKEIQRLRDQLKARYSTTTLLEQLEETTREGERREQV LKALSEEKDVLKQQLSAATSRIAELESKTNTLRLSQTVAPNCFNSSINNIHEMEIQL	302
3	FGFR2-WAC	MDLVSEMEMMKMIGKHKNIINLLGACTQDGPLYVIVEYASKGNLREYLRARRPPGMEYSYD INRVPEEQMTFKDLVSCTYQLARGMEYLASQKCIHRDLAARNVLVTENNVMKIADFGLARD INNIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPYPGIPVEE LFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLTTNE|ASRL REEAHNMGTIHMSEICTELKNLRSLVRVCEIQATLREQRILFLRQQIKELEKLKNQNSFMV	305
4	FGFR2-WAC	MGLARDINNIDYYKKTTNGRLPVKWMAPEALFDRVYTHQSDVWSFGVLMWEIFTLGGSPY PGIPVEELFKLLKEGHRMDKPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLT TNE|PYDSADDWSEHISSSGKKYYYNCRTEVSQWEKPKEWLEREQRQKEANKMAVNSFP KDRDYRREVMQATATSGFASGMEDKHSSDASSLLPQNILSQTSRHNDRDYRLPRAETHS SSTPVQHPIKPVVHPTATPSTVPSSPFTLQSDHQPKKSFDANGASTLSKLPTPTSSVP	296

"M" delineates addition of a methionine (i.e., start codon after translation) to allow transcription and translation of the entire minigene sequence; "|" delineates the fusion breakpoint

Supplementary Table 6. Sequence of the FGFR2 fusion-reactive TCR from patient 1

TCR chain	V region	J region	CDR3b amino acid sequence
TCRA	TRAV9-2	TRAJ9	CALSDRNTGGFKTIF
TCRB	TRBV5-1	TRBJ2-7	CASSLPNSSFFTYEQYF