Comprehensive gene expression analysis of *IDH1/2* mutant biliary cancers (BC)

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Introduction

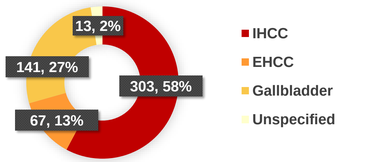
- Isocitrate dehydrogenases (IDH) mutations identify a distinct subtype of BC that has yet to be fully characterized.
- We recently showed that *IDH1/2* mutant BC harbor specific gene alterations involving chromatin remodeling and DNA repair, and a differential immune markers profile compared to other *IDH* mutant gastrointestinal tumors [1].
- Here we aim to further dissect the molecular profile of IDH mutant BC through a comprehensive gene expression profiling analysis.

Methods

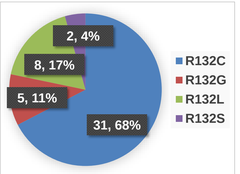
- 524 BC samples (303 intrahepatic cholangiocarcinoma, IHCC, 67 extrahepatic cholangiocarcinoma, EHCC, 141 gallbladder, 13 unspecified) collected between February to December of 2019 were included in the analysis.
- Samples were analyzed using NextGen DNA sequencing (NextSeq, 592 gene panel), whole transcriptome RNA sequencing (NovaSeq) and immunohistochemistry (Caris Life Sciences, Phoenix, AZ).
- EBseq was used to identify differentially expressed genes in *IDH* mutant vs wild type (WT) tumors with control for false discovery rate (FDR, $Q < 0.2$).
- Pathway and functional enrichment analysis was performed using g:Profiler and Enrichr.
- Microenvironment Cell Population-counter (MCP-counter) was used for quantification of the abundance of immune and stromal cell population using transcriptomic data [2].

1. Battaglin et al. J Clin Oncol, 2020. 2. Becht et al. Genome Biology, 2016.

Figure 1. Study Population.



Pathogenic *IDH1* Mutations



Pathogenic *IDH2* Mutations

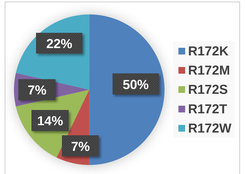


Figure 2. *IDH1/2* Mutation Frequency.

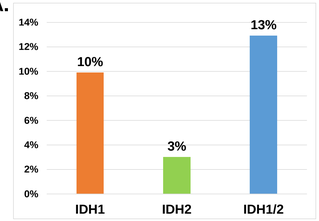


Table 1. Patient Demographics.

Mutational Status	FEMALE	MALE	MEDIAN AGE (range)
***IDH* WT**	237	227	64.9 (26-91)
***IDH1* Mut**	32	14	64.3 (35-84)
***IDH2* Mut**	11	3	61.1 (26-91)

- *IDH* mutation was more common in females ($P = 0.0036$).
- No significant association with age was observed.

Figure 3. Mutational Profiles of *IDH1/2* Mutant vs WT.

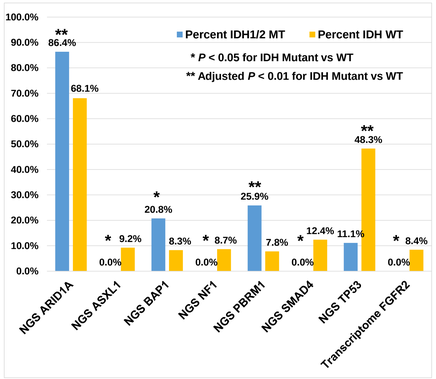
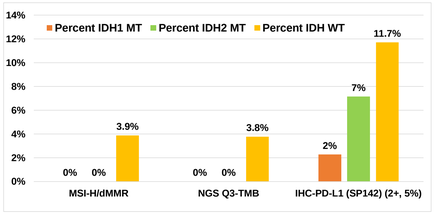


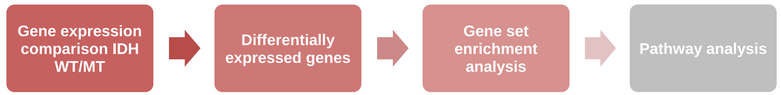
Figure 4. Immune Checkpoint Related Markers According to *IDH1/2* Status.



TMB cutoff ≥17 mt/MB.
MSI-H/dMMR status determined by IHC, Fragment analysis and NGS.

Gene Expression Analyses Workflow.

- **A total of 774 genes were significantly differentially expressed between *IDH* mutant and WT: 582 underexpressed (Fold change, FC: 0.025~0.699); 192 overexpressed (FC: 1.43~3.3).**



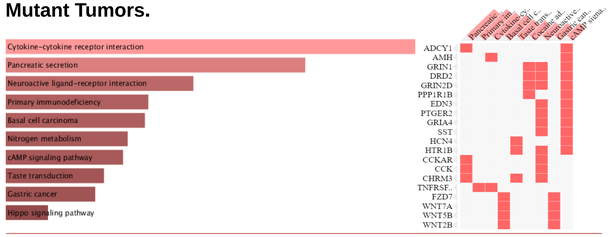
Results

Figure 5. Hallmarks of Cancer Evaluation via g:Profiler of Differentially Expressed Genes in *IDH* Mutant and WT Tumors.

Gene Sets	Adjusted *P*-value *	Enrichment Score	Term Size	Query Size	Intersection Size
INFLAMMATORY_RESPONSE	0.0048	2.313	200	143	17
KRAS_SIGNALING_DN	0.0049	2.313	200	143	17

* only significant results are shown.

Figure 6. KEGG Analysis of Underexpressed Genes in *IDH* Mutant Tumors.



Term Name	ID	Adjusted *P*-value	Negative log10 Adj *P*-value	Term Size	Query Size	Intersection Size
Cytokine-cytokine receptor interaction	KEGG:04060	0.0023	2.6357	292	284	26
Pancreatic secretion	KEGG:04972	0.034	1.464	102	284	12

Figure 8. WikiPathways Gene Set Evaluation via g:Profiler of Differentially Expressed Genes in *IDH* Mutant and WT Tumors.

Term Name	ID	Adjusted *P*-value *	Negative log10 Adj *P*-value	Term Size	Query Size	Intersection Size
Cancer immunotherapy by PD-1 blockade	WP:WP4585	0.020	1.681	23	244	6

* only significant results are shown.

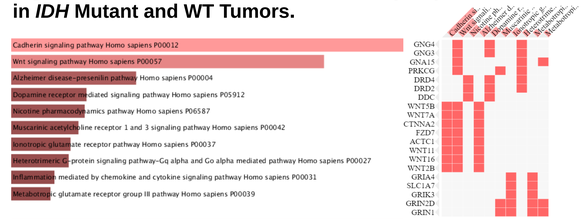
Cancer immunotherapy by PD-1 blockade		FC MT/WT
IFNG	interferon gamma	**0.32**
CD8B	CD8b molecule	**0.37**
BATF	basic leucine zipper ATF-like transcription factor	**0.40**
PDCD1	programmed cell death 1	**0.53**
LCK	LCK proto-oncogene, Src family tyrosine kinase	**0.55**
PDCD1LG2	programmed cell death 1 ligand 2	**0.61**

Conclusions

- Our data show for the first time a distinct gene expression profile characterizing *IDH* mutant tumors which display significant downregulation of inflammatory response pathways and immune-related genes, coupled with significantly lower B cell infiltration and higher endothelial abundance.
- These findings contribute to further the understanding of *IDH* mutant BC and may inform the future development of rational combination therapies.

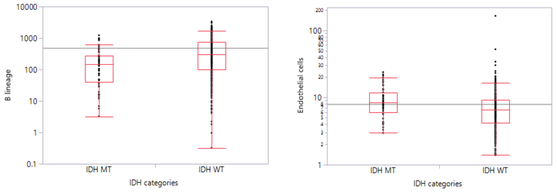
HALLMARK INFLAMMATORY RESPONSE		FC MT/WT
ROS1	ROS proto-oncogene 1, receptor tyrosine kinase	**0.12**
IL1A	interleukin 1 alpha	**0.20**
NDP	norrin cystine knot growth factor NDP	**0.22**
CSF3	colony stimulating factor 3	**0.24**
OSM	oncostatin M	**0.30**
CD70	CD70 molecule	**0.40**
GNA15	G protein subunit alpha 15	**0.41**
RGS16	regulator of G protein signaling 16	**0.51**
IRAK2	interleukin 1 receptor associated kinase 2	**0.52**
LCK	LCK proto-oncogene, Src family tyrosine kinase	**0.55**
SELE	selectin E	**0.55**
PTGER2	prostaglandin E receptor 2	**0.56**
MEFV	MEFV innate immunity regulator, pyrin	**0.58**
CCR7	C-C motif chemokine receptor 7	**0.58**
KCNA3	potassium voltage-gated channel subfamily A member 3	**0.63**
SLAMF1	signaling lymphocytic activation molecule family member 1	**0.67**
SCN1B	sodium voltage-gated channel beta subunit 1	**1.43**

Figure 7. Panther Analysis of Differentially Expressed Genes in *IDH* Mutant and WT Tumors.



Panther 2016	Overlap	*P*-value	Adjusted *P*-value
Cadherin signaling pathway Homo sapiens_P00012	24/150	3.45E-09	3.86E-07
Wnt signaling pathway Homo sapiens_P00057	31/278	1.32E-07	7.40E-06

Figure 9. MCP Counter Results in *IDH* Mutant vs WT Tumors.



- Significantly lower B cell infiltration and higher endothelial abundance in MT tumors.