Tumor profiling of biliary tract carcinomas reveals distinct molecular alterations and potential therapeutic targets

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Abstract

Background: Extrahepatic cholangiocarcinoma (EHCC), intrahepatic cholangiocarcinoma (IHCC), and gallbladder carcinoma (GBCA) are rare tumors with poor prognosis that tend to be chemo-resistant. The underlying molecular alterations and their correlation with altered responses to therapies are not well understood. We hypothesized that delineation of different molecular alterations in the cancer types might potentially yield different therapeutic options.

Methods: 815 cases (126 EHCC, 434 IHCC, 244 GBCA, 11 NOS) were tested by a commercial multiplatform profiling service (Caris Life Sciences, Phoenix, AZ). Tests included sequencing (Sanger, NGS), gene amplification (CISH/FISH), and protein expression (IHC).

Results: 24 of 47 genes tested had mutations, with the highest rates in TP53 (28%), KRAS (18%), IDH1 (9%), and SMAD4 (6%). BRCA1/2 mutations were seen in 3/41 (7.3%) and 5/40 (12.5%) cases. Overall, IHC showed high TOPO1, TOP2A, PD-1, SPARC and PD-L1 in 56%, 49%, 40%, 39% and 15% of cases and low RRM1, ERCC1 and TS in 82%, 72% and 79%, respectively, suggesting potential utility of chemotherapeutic and immunomodulatory agents targeting these alterations in selected cases. Mutually exclusive protein loss of chromatin modifiers BAP1 and PBRM1 were seen in 17% and 27%. ROS1 break-apart FISH showed negative results in 16 cases tested.

Comparing the three carcinomas (EHCC, IHCC and GBCA, Table), EHCC had the highest KRAS mutation rate; IHCC had the highest IDH1 mutation rate; GBCA and EHCC had significantly higher TP53 mutation rates and HER2 amplification than IHCC. IDH1 and TP53 mutations were mutually exclusive, and IDH1-mutated IHCC had higher P-glycoprotein expression than TP53-mutated IHCC (82% vs. 37%, P<0.01). GBCA had high TOP2A by FISH and IHC, and a high loss of PBRM1.

	EHCC %	IHCC %	GBCA%
KRAS	28	17	13
IDH1	0	14	1.5
TP53	44	8	41
Her2 FISH	18	1.5	15
TOP2A FISH	0	0	21
TOP2A IHC	43	39	71
PBRM1 Loss IHC	15	21	53

Conclusion: Multiplatform cancer profiling reveals distinct biomarker characteristics of biliary tract carcinomas, offering insights into disease biology and suggests potential sensitivity to novel and conventional therapies. Further analyses with clinical correlation are warranted.

Results

Table 1: Patient Characteristics

Cancer types	Case N	Case with NGS N	Average Age (Range)
IHCC (Intrahepatic cholangiocarcinoma)	434	120	58.3 (20-87)
EHCC (Extrahepatic cholangiocarcinoma)	126	25	63 (27-85)
GBCA (Gallbladder cancer)	244	64	64 (34-91)
Bile duct cancer, NOS	11	1	58.5(43-70)
Overall	815	210	60.6 (20-91)

Figure 1: Gene mutations found in 47 genes sequenced using a combination of NextGen (Illumina MiSeq platform) and Sanger sequencing.

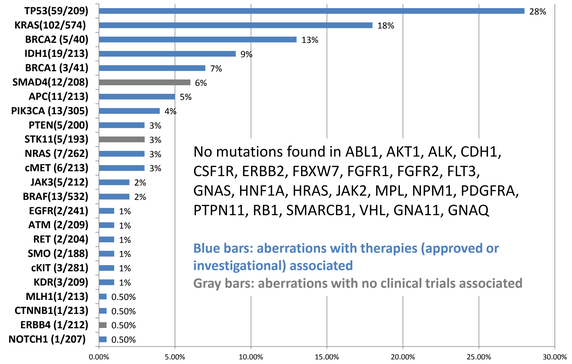


Figure 2: Fluorescent/chromogenic *in situ* hybridization reveals gene amplification frequencies in the complete cohort. Previous reports have shown conflicting results on presence of ROS1 rearrangement in cholangiocarcinoma (1,2). Our data on 16 cases showed no ROS1 rearrangement using Cytocell ROS1 (6q22) break-apart probes.

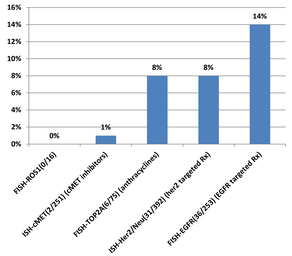
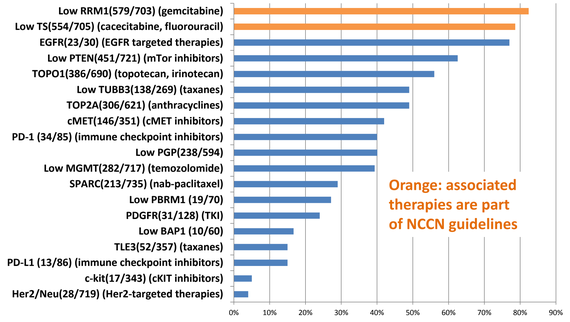


Figure 3: Distribution frequencies of immunohistochemistry markers and associated therapies in the complete cohort (N=815). Standard-of-care therapies as well agents not routinely considered for biliary tract cancers are suggested by corresponding predictive biomarkers.



PD-1 (Ab. MRQ-22) expression was measured on Tumor Infiltrating Lymphocytes (TIL) with the cutoff of 1+,1%; PD-L1 (Ab. 130021) expression was measured on tumor cells with the cutoff of 2+, 5%. Antibody information on other markers available upon request.

Figure 4: Biomarker differences between IHCC, EHCC and GBC cohorts. Statistically significant differences are seen in 13 biomarkers investigated among the three cohorts. Left boxes show therapeutic agents potentially suggested by the biomarker aberrations or the biological functions of the biomarker, while the therapeutic implications are still being investigated. Connective lines indicate statistical significance (p<0.05)

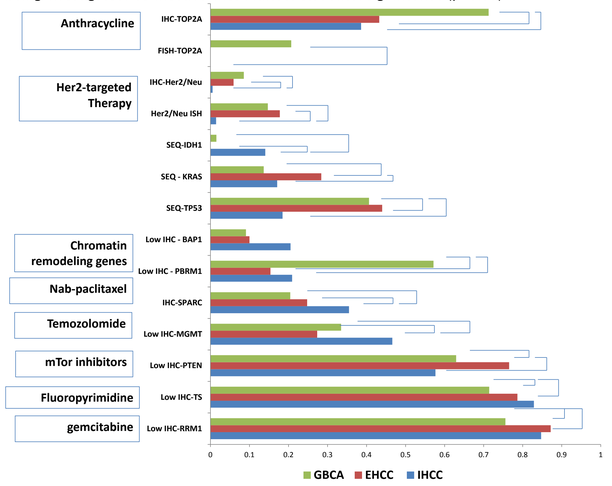
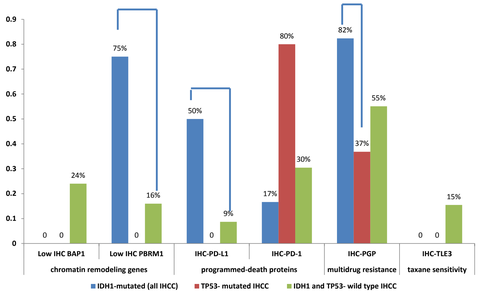


Figure 5: Subgroup analysis in IHCC. IDH1 mutations occur exclusively in IHCC and is mutually exclusive of TP53, suggesting these mutations may be independent oncogenic drivers for IHCC. Immunohistochemistry reveals different protein expressions in IDH1-mutated (N=17); TP53-mutated (N=22) and double-wild type (N=80)cohorts. No significant differences were seen by ISH (*in situ* hybridization) or sequencing. Connective lines indicate statistical significance (p<0.05)



Conclusions

- Multiplatform cancer profiling reveals distinct biomarker characteristics of biliary tract carcinomas, offering insights into disease biology and suggests potential sensitivity to novel and conventional therapies.
- GBCA is characterized by high TOP2A expression and gene amplification as well as a high loss of the chromatin remodeling protein PBRM1.
- EHCC shows a significantly higher KRAS mutation rate than IHCC and GBCA
- Her2 protein expression and gene amplification are significantly higher in GBCA and EHCC compared to IHCC, suggesting Her2-targeted therapy.
- IHCC is characterized by IDH1 mutations. The highest SPARC expression and the lowest MGMT expression are seen in IHCC.
- IDH1 and TP53 mutations are mutually exclusive; different protein expression patterns observed in IDH1-driven and TP53-driven IHCC suggest that further molecular subgroups may exist within IHCC, highlighting the need for a multiplex tumor profiling on individual tumors.

References

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2. Liu, P et al. (2011) ROS kinase fusions are not common in Chinese patients with cholangiocarcinoma J South Med Univ, 2013, 33(4): 474-478