Comprehensive multiplatform biomarker analysis of 313 hepatocellular carcinoma identifies potential novel therapeutic options

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Abstract

Background: Effective treatment strategies for hepatocellular carcinoma (HCC) remain limited. Identification of additional therapies remains paramount as currently available agents have resulted in marginal improvements in overall survival.

Methods: 313 HCC samples were evaluated using a multiplatform profiling service (Caris Life Sciences, Phoenix, AZ), including gene sequencing (Sanger, NGS [N=79]), protein expression (IHC) and gene amplification (ISH).

Results: Biomarker changes of interest are shown.

Percentage of samples with change in protein expression, by IHC									
High expression levels						Low expression levels			
EGFR	TOPO1	PD-1	TOP2A	SPARC	cMET	RRM1	TS	PTEN	MGMT
58	52	52	36	35	25	82	80	66	32

TP53 was mutated in 28%, CTNNB1 in 23%, and BRCA2 in 20%; other gene mutation rates were < 5%.TP53-mutated tumors show significantly higher TOP2A (89% vs. 39%, p<0.0001), TS (70% vs. 32%, p=0.0067) and RRM1 expression (40% vs. 12%, p=0.017), implying high rates of proliferation and DNA synthesis. CTNNB1-mutated tumors showed significantly higher SPARC (67% vs. 21%, p=0.0013) and AR expression (53% vs. 22%, p=0.025).
Metastatic HCC (N=112) exhibited significantly higher PD-1 (89% vs. 33%, p=0.0128) and TS expression (35% vs. 13%, p<0.0004) than non-metastatic (N=201).

Conclusions: These data suggest potential therapeutic targets, such as tyrosine kinase inhibitors, anti-PD1 agents, or PI3 kinase pathway inhibitors. Although no evidence shows that cytotoxics are effective in patients with HCC, irinotecan, alkylating agents, fluoropyrimidines, anthracyclines, nab-paclitaxel, gemcitabine, or taxanes may be therapeutically relevant. The protein changes associated with CTNNB1-mutated tumors suggest potential benefit of targeting WNT pathway in combination with nab-paclitaxel or anti-androgens. Immuno-modulatory agents may be a therapeutic option in metastatic HCC, based on the higher levels of PD-1.

Multiplatform tumor profiling reveals molecular heterogeneity of HCC, similar overall to previous reports, and identifies different potential treatment options for molecular subtypes.

Demographics

Table 1. Patient demographics. Includes limited documented information of risk factors obtained on a very small subset of cases.

Median age: 61
Age range: 12-87
M:F ratio=2.7:1
Known mets: 36%
Known Viral status: 3=HBV+, 8=HCV+
Known EtOH: 5

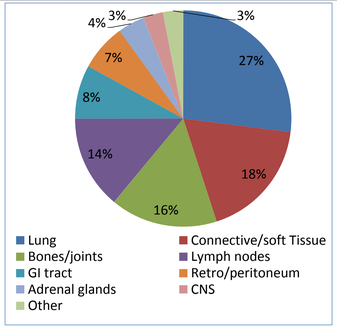
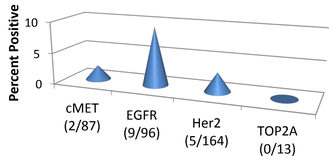


Figure 1 – Sites of Metastases.

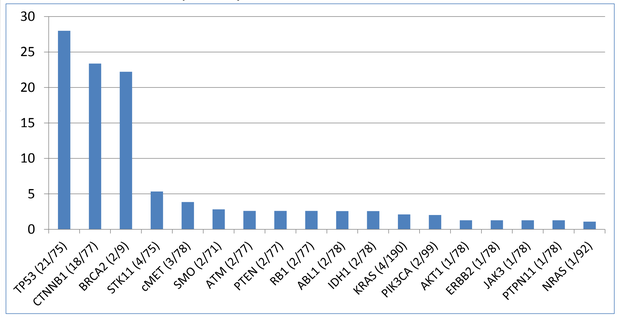
Results, FISH

Figure 1. **Changes in gene copy** number as measured by FISH or CISH were identified for cMET, EGFR, and HER2. No changes were seen for TOP2A.



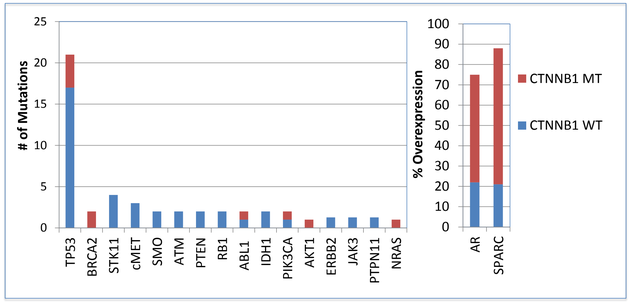
Results, Gene Mutations

Figure 3. Gene alterations. Mutations were found in only 38% of 47 genes tested. Genes with no alterations identified included ALK, APC, BRAF, CDH1, c-KIT, CSF1R, EGFR, ERBB4, FBXW7, FGFR1, FGFR2, FLT3, GNA11, GNAQ, GNAS, HNF1A, HRAS, JAK2, KDR, MLH1, MPL, NOTCH1, NPM1, PDGFRA, RET, SMAD4, SMARCB1, VHL, and BRCA1. 47% of cases tested had either a CTNNB1 or a TP53 gene alteration, including 4 cases with both a CTNNB1 and a TP53 gene alteration. All CTNNB1 alterations were in exon 3. 2 of the 4 KRAS alterations were Q61H. No significant differences in gene mutations were found between primary and metastatic cases.



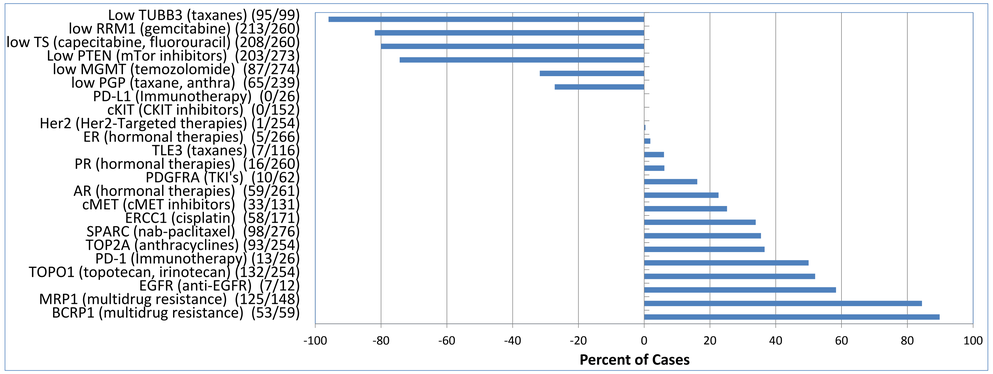
Results, Co-incidence

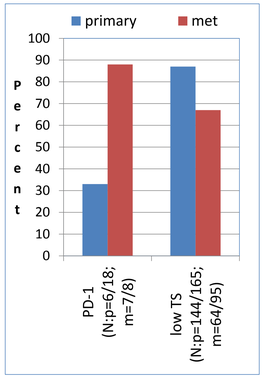
Figure 4. Incidence of gene alterations/ changes in protein expression in the presence or absence of CTNNB1 pathway alteration. Cases that were CTNNB1 wild type (WT, 59) vs. cases with CTNNB1 mutations (MT, 18) were compared for incidence of gene alterations. Significant changes in expression of SPARC and AR were also noted (other changes in protein expression were not significant).



Results, Immunohistochemistry (IHC)

Figure 2. Levels of protein expression, A. either overexpression, reported as percent positive of total cases tested, or loss, reported as percent negative (except for PD-1: PD-1+ tumor infiltrating lymphocytes). **B.** Comparison of protein expression, for those with significant differences between primary and metastatic cases (PD-1, p=0.0128; TS, p=0.0004).





Conclusions

- Data presented herein and suggestions for therapeutic potential are limited by the lack of clinical outcomes from the analysis.
- These data suggest potential therapeutic targets, such as tyrosine kinase inhibitors, anti-PD1 agents, or PI3 kinase pathway inhibitors. Although no evidence shows that cytotoxics are effective in patients with HCC, irinotecan, alkylating agents, fluoropyrimidines, anthracyclines, nab-paclitaxel, gemcitabine, or taxanes may be therapeutically relevant in a selected population.
- The protein changes associated with CTNNB1-mutated tumors suggest potential benefit of targeting WNT pathway in combination with nab-paclitaxel or anti-androgens.
- Significantly higher PD-1+ tumor infiltrating lymphocytes and TS expression in the metastases compared to primary HCC may suggest increased opportunity for immune checkpoint inhibitors in the metastases and higher likelihood of fluoropyrimidine agents to be effective in the primary tumors.

References

1. Tornesello, M et al. Mutations in TP53, CTNNB1 and PIK3CA genes in hepatocellular carcinoma associated with hepatitis B and hepatitis C virus infections , *Genomics* 102, 2013: 74-83.
2. Li, L and Mao, M. Next generation sequencing reveals genetic landscape of hepatocellular carcinomas, *Cancer Letters* 340, 2013: 247–253.