Epigenetic Regulation of Combined Hepatocellular-Cholangiocarcinoma Subtypes

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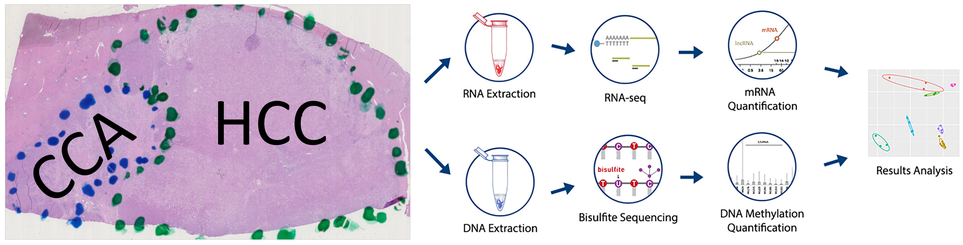
Poster Number: 4336

Introduction

- Combined hepatocellular-cholangiocarcinoma (HCC-CCA) is a rare liver tumor comprising histologic features of both HCC and CCA.
- Due to its heterogeneous nature, treatment of combined HCC-CCA is a significant clinical challenge and prognosis remains poor.
- Further understanding of the tumor biology underlying the individual subtypes of this mixed tumor is required to improve treatment stratification and optimize treatment strategies.
- This study sought to identify epigenetic regulation underlying gene expression patterns in the individual components of combined HCC-CCA.

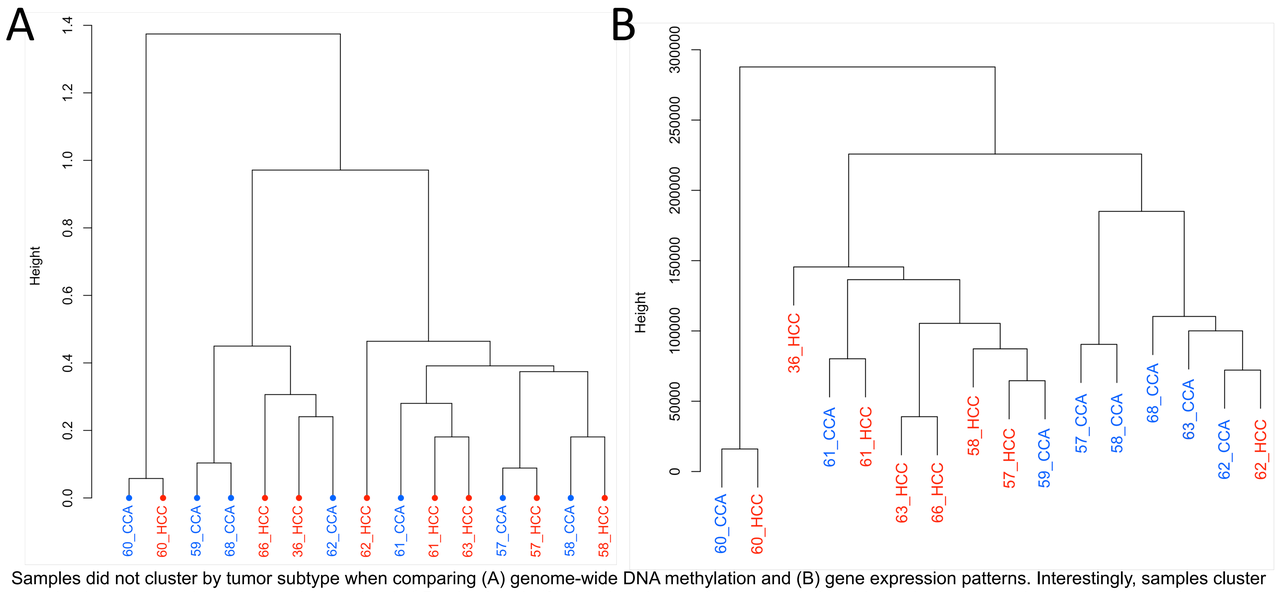
Materials and Methods

- Formalin fixed paraffin embedded tumor specimens from 10 patients diagnosed with combined HCC-CCA were utilized in this study.
- Hematoxylin and eosin staining was performed for each sample, and regions representative of the individual HCC and CCA components were delineated by a human pathologist specializing in liver malignancies.
- Unstained slides were cut and dissected to separate HCC and CCA components.
- DNA and RNA extraction was performed for each sample for DNA methylation (n = 8 HCC and 7 CCA) and gene expression (n = 8 HCC and 8 CCA) profiling via reduced representation bisulfite sequencing and RNA-seq, respectively.



Results

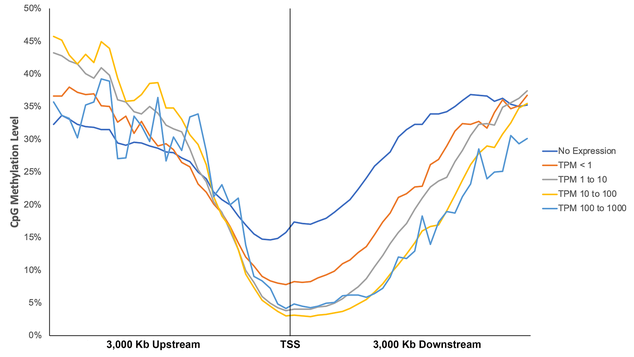
Figure 1. Genome-Wide DNA Methylation and Gene Expression Clustering



Samples did not cluster by tumor subtype when comparing (A) genome-wide DNA methylation and (B) gene expression patterns. Interestingly, samples cluster by patient as opposed to cancer subtype in 4 out of 5 patients with DNA methylation profiles available for both subtypes, suggesting similar epigenetic regulatory patterns arising from development in the same microenvironment and genetic background. A similar phenomenon was observed for gene expression profiles, with 3 out of 6 pairs clustering by patient as opposed to tumor subtype.

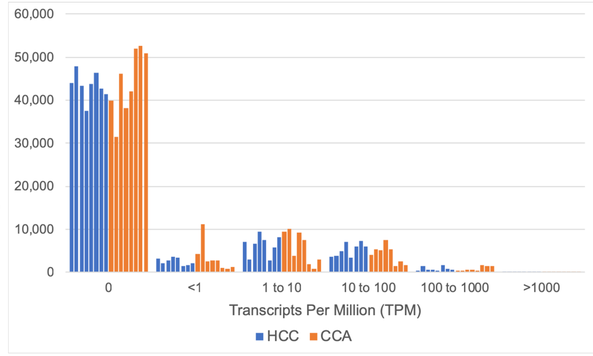
Results (cont.)

Figure 2. DNA Methylation Correlated with Gene Expression at Transcription Start Sites



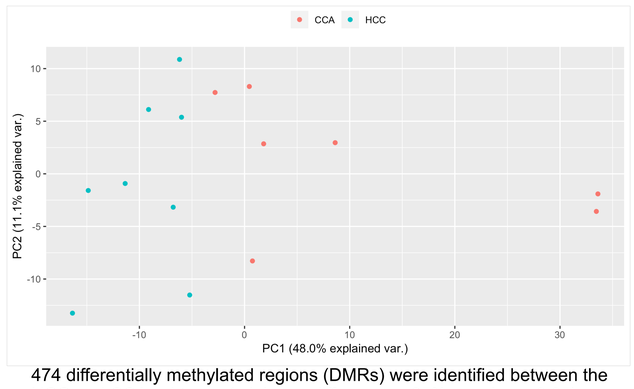
As expected, DNA methylation levels at transcription start sites (TSS) were negatively correlated with gene expression in all samples (Spearman's rho = -0.133 to -0.546; $p < 2.2 \times 10^{-16}$).

Figure 3. High Number of Unexpressed Genes Suggesting RNA Degradation in FFPE Samples



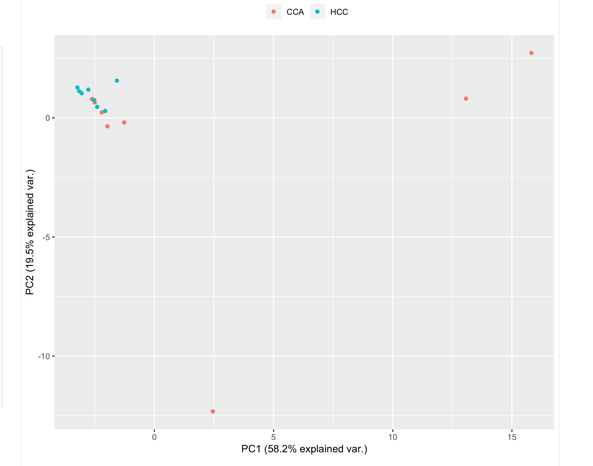
High numbers of genes displayed no expression in each samples (average 43,765/sample), suggesting reduced library diversity due to RNA degradation in FFPE samples.

Figure 4. Differential Methylation Differentiates HCC-CCA Subtypes



474 differentially methylated regions (DMRs) were identified between the HCC and CCA subtypes (minimum difference > 25%; q-value < 0.05). Tumor specific clustering demonstrates HCC-CCA subtypes can be differentiated based on DNA methylation patterns.

Figure 5. Samples do not Cluster by Tumor Type Based on Differentially Expressed Genes



58 differentially expressed genes (DEGs) were identified between the HCC and CCA subtypes (q-value < 0.05). Lack of tumor specific clustering suggests RNA degradation in FFPE samples.

- DMRs overlapped with 324 known genes, 1 of which (*STK38L*) displayed increased expression (log2 fold change = 4.39; q-value = 0.01) associated with hypomethylation of 2 regions (-34.86% and -27.47%; q-value < 1×10^{-38}) in the CCA compared to HCC group.
- *STK38L* encodes a serine/threonine kinase involved in Hippo signaling, a highly conserved signaling pathway that functions as a key coordinator of tissue growth and homeostasis.

Conclusions and Future Work

- These results demonstrate epigenetic patterns can differentiate individual components of combined HCC-CCA.
- Subtype specific circulating tumor DNA methylation patterns could be quantified in blood as minimally invasive biomarkers.
  - Early detection
  - Response to therapy for individual subtypes.
- RNA degradation reduces usability of FFPE samples for transcriptional analysis.
- Future prospective studies may aim to confirm these results and identify subtype specific biomarkers to improve detection and prognosis for this deadly disease.

Funding: Illinois Health Disparities Initiative