Molecular and Immunohistochemical Analysis of Mucinous Cystic Neoplasm of the Liver

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ABSTRACT

Objectives: Mucinous cystic neoplasm of the liver is characterized by neoplastic mucinous and/or biliary epithelium surrounded by ovarian-type stroma. Immunohistochemical studies have shown that the ovarian-type stroma expresses estrogen receptor, suggesting potential hormonal responsiveness. The molecular biology of mucinous cystic neoplasm of the liver remains poorly studied.

Methods: Transcriptome sequencing and immunohistochemistry were performed on a series of mucinous cystic neoplasms.

Results: Mucinous cystic neoplasm of the liver exhibited significantly increased RNA expression of ovarian stromal markers WT1, PR, and ER2 and sex cord stromal markers SF-1, inhibin- α , and calretinin compared with nonneoplastic liver. Immunohistochemistry confirmed the RNA-level data. Evidence for sex hormone biosynthesis was identified by significant overexpression of multiple estrogen biosynthetic enzymes. Expression of 17 β -hydroxysteroid dehydrogenase 1 was confirmed immunohistochemically. Pathway analysis also identified significant upregulation of the hedgehog and Wnt pathways and significant downregulation of T-helper 1 and T-helper 2 pathways.

Conclusions: Mucinous cystic neoplasm of the liver recapitulates ovarian stroma at the morphologic, DNA, RNA, and protein levels. These data support the concept that this tumor likely arises from ectopic primitive gonadal tissue and/or stromal cells with capacity to transdifferentiate to ovarian cortical cells.

Key Points

- The stroma of mucinous cystic neoplasm not only resembles ovarian stroma but also shows features of hormone production.
- Estrogen may have an important role in biliary neoplasia biology.
- Mucinous cystic neoplasm is a hedgehog pathway-activated tumor.

Mucinous cystic neoplasm of the liver, previously referred to as hepatobiliary cystadenoma with mesenchymal stroma, was first classified as a distinct tumor type by Wheeler and Edmondson in 1985.¹ The pancreatic counterpart of mucinous cystic neoplasm of the liver is designated mucinous cystic neoplasm of the pancreas.² Mucinous cystic neoplasm of the liver is rare, comprising less than 5% of all hepatic cystic neoplasms and diagnosed virtually exclusively in women in the fourth or fifth decade of life.^{2,3} The histopathology of mucinous cystic neoplasm of the liver is distinct, exhibiting a cystic lining composed of columnar to cuboidal epithelial cells with mucin overlying ovarian-type stroma.^{1,3-7} Some cases have variable amounts of mucinous and nonmucinous or biliary epithelium. This feature has led some to postulate that mucinous cystic neoplasm of the liver undergoes progression from nonmucinous or biliary epithelium to mucinous epithelium, at which point dysplastic change may occur.⁸ Nonetheless, the transition to carcinoma in situ or invasive adenocarcinoma is an uncommon event in mucinous cystic neoplasm of the liver, with a rate of malignant evolution between 3% and 6% in some case series.^{4,5}

Immunohistochemical investigation of mucinous cystic neoplasm of the liver has ascertained that the ovarian-type stroma is positive for progesterone receptor, estrogen receptor, and inhibin- α .^{3,9-11} These findings suggest that the stroma of mucinous cystic neoplasm of the liver is potentially hormonally responsive similar to normal ovaries. A study by Westerhoff et al¹² showed that FOXL2, a transcription factor expressed in normal ovaries, is also expressed in the ovarian-type stroma of mucinous cystic neoplasm of the liver; however, the role of FOXL2 in mucinous cystic neoplasm of the liver remained to be elucidated.

Prior studies have evaluated the molecular biology, gene expression profile, and immunohistochemical phenotype of pancreatic mucinous cystic neoplasms, which are histologically similar to mucinous cystic neoplasms of the liver. Therefore, discussion and comparison of pancreatic mucinous cystic neoplasm and hepatic mucinous cystic neoplasm are important. The ovarian-like stroma of pancreatic mucinous cystic neoplasms has been proposed to be hormonally responsive based on estrogen receptor expression by immunohistochemical and gene expression analysis similar to liver mucinous cystic neoplasms.^{11,13} Immunohistochemistry-based studies of the ovarian-like stroma of pancreatic and hepatic mucinous cystic neoplasms have also alluded to its potential steroidogenic capability with expression of StAR, CYP17, CYP19, 17 β -hydroxysteroid dehydrogenase 1, 3 β -hydroxysteroid dehydrogenase, and CYP11.^{11,14} However, gene expression analysis of steroidogenic enzymes has never been performed in either pancreatic or hepatic mucinous cystic neoplasms. Elucidation of the molecular pathways driving the histogenesis of the ovarian-like stroma in pancreatic mucinous cystic neoplasms have shown overexpression of the WNT/ β -catenin pathway based on expression of some members of the WNT/ β -catenin pathway such as secreted Frizzled-related protein, WNT1, FZD3, FZD6, and nuclear β -catenin accumulation in the ovarian-like stroma.^{11,13,15} Furthermore, some studies have identified inactivating mutations in *RNF43*, an ubiquitin ligase and a negative regulator of the WNT/ β -catenin pathway.¹⁶ The epithelial component of pancreatic mucinous cystic neoplasms has also been investigated by whole-exome sequencing and targeted sequencing demonstrating mutations in *KRAS*, *p53*, *SMAD4*, *p16*, *RNF43*, and *PIK3CA*.¹⁶⁻¹⁹ *KRAS* mutations appear to occur more commonly early in pancreatic mucinous cystic neoplasms with low-grade dysplasia, whereas *p53* and *SMAD4* mutations present late, usually occurring in high-grade or malignant transformation.^{16,17} Mucinous cystic neoplasms of the liver also appear to possess

KRAS mutations but at a lower frequency than pancreatic mucinous cystic neoplasms and not typically in low-grade dysplasia.²⁰ Epigenetic analysis has also found hypermethylation of *p14* and *p16* in noninvasive pancreatic mucinous cystic neoplasms.²¹

Currently, no comprehensive molecular studies of mucinous cystic neoplasm of the liver have been performed. Our goal was to elucidate the molecular biology of mucinous cystic neoplasm of the liver focusing on the ovarian-type stroma, using DNA and RNA sequencing along with immunohistochemical confirmation.

Materials and Methods

Specimen Selection

After institutional review board approval by multiple institutions, cases of mucinous cystic neoplasm of the liver diagnosed between 2007 and 2017 with low-grade dysplasia in partial hepatectomy specimens were procured retrospectively from the following institutions: Mayo Clinic, Cleveland Clinic, Emory University, University of California Los Angeles, and University of Washington (M.W. was previously affiliated with this institution). All H&E and immunohistochemically stained slides from each case were retrospectively reviewed by pathologists with gastrointestinal or liver subspecialty training at each respective institution to confirm the diagnosis of mucinous cystic neoplasm of the liver, to select a representative tumor block, and to identify a nonneoplastic liver tissue block for downstream studies. The goal of this study was to perform a comprehensive molecular and immunohistochemical investigation of the ovarian-like stroma of mucinous cystic neoplasm of the liver. Therefore, extensive clinical data were not examined.

Transcriptomic Analysis and Molecular Pathway Analysis

Nine mucinous cystic neoplasms of the liver and corresponding nonneoplastic pairs were selected for RNA sequencing (RNAseq) using Illumina's TruSeq RNA Access Library Prep. Three of the cases were excluded because they failed RNA extraction, likely because of RNA degradation before tissue fixation. After exclusion, a total of 6 cases were examined by RNAseq and processed using MAP-RSeq v3.0.0, which uses STAR v2.6.1 and the reference human genome hg38.^{22,23} Gene counts were calculated using feature counts v1.5.3, and differential expression analysis was performed using edgeR 2.6.2.^{24,25} Genes with an absolute fold change greater than 2-fold and a false

discovery rate less than 0.10 were selected for pathway enrichment analysis using Ingenuity pathway analysis and Ingenuity Systems pathways.

Next-Generation DNA Sequencing

The Human Comprehensive Cancer Panel (DHS-3501Z) from Qiagen was used for next-generation sequencing on 19 patients with mucinous cystic neoplasm of the liver and adjacent normal tissue. An additional 3 patients with only tumor tissue were also included for sequencing.

Mutational Analysis

The Qiagen Gene Globe Data Portal was used for analysis of the samples. Variant calling was performed using smCounter, Qiagen's molecular tag-aware variant-calling algorithm. After variant calling, we performed an additional filtering step to identify potential somatic variants by (1) removing variants that did not pass the default filtering defined in the software (eg, variant molecular tag depth, strand bias, base quality, etc); (2) excluding non-protein-coding variants and variants that do not belong to the following categories: missense/stop_retained/inframe/frameshift; (3) excluding variants that are annotated in the Single Nucleotide Polymorphism Database; (4) excluding variants with frequency less than 0.25 and a molecular tag of 5 or less; (5) excluding variants with recurrence among samples smaller than 2 occurrences; and (6) excluding variants observed in paired normal tissue.

Immunohistochemistry

Immunohistochemistry with commercially available antibodies was performed on formalin-fixed, paraffin-embedded tissue sections of mucinous cystic neoplasm of the liver tumor blocks selected for the following proteins: estrogen receptor (Leica; monoclonal 6F11, 1:300) and keratin 19 (Dako; monoclonal RCK108, 1:200). The tissue blocks were sectioned at 4 μ m, deparaffinized, and processed on the Ventana BenchMarkXT immunohistochemical stainer (Ventana; Roche Diagnostics). Following processing, the tissue sections were counterstained with hematoxylin. In addition, a subset of 13 mucinous cystic neoplasms of the liver with available residual material were immunostained, using the same methodology as above, with commercially available antibodies using formalin-fixed, paraffin-embedded tissue sectioned at 4 μ m for the following antibodies: SF-1 (Thermo Fisher; N1665), progesterone receptor (Dako; monoclonal

PgR363, 1:800), calretinin (Leica; monoclonal CAL6, prediluted), inhibin- α (Ventana [Cell Marque]; monoclonal R1, prediluted), melan-A (Leica; monoclonal A103, prediluted), WT-1 (Leica; monoclonal WT49, prediluted), 17 β -hydroxysteroid dehydrogenase 1 (Sigma-Aldrich; polyclonal, 1:300), and GLI1 (Santa Cruz; monoclonal C-1). Immunostains were scored as positive if greater than 5% of the cells stained.

Results

Stroma of Mucinous Cystic Neoplasms of the Liver and Ovarian Stroma Demonstrate Similarities in Transcriptome, Morphology, and Immunophenotype

Morphologically, the stroma of all mucinous cystic neoplasms of the liver ($n = 22$) resembles stroma of the normal ovary, with stromal cells exhibiting spindled morphology and scant cytoplasm, which is a well-known finding in mucinous cystic neoplasms of the liver. On occasion, luteinized stromal cells can be seen in the stroma, as was focally seen in all cases [Image 1](#). In addition, occasional steroidogenic cells with vacuolated cytoplasm histologically similar to cells of the adrenal cortex or the ovary can also be seen in the stroma of mucinous cystic neoplasm of the liver. The mucinous epithelium is characterized by a single layer of mucin filled cuboidal to columnar cells, each with a basally oriented nucleus. However, some mucinous cystic neoplasms of the liver display nonmucinous biliary-type epithelium mixed with mucinous epithelium. The epithelium also stained diffusely strong with keratin 19 in all cases tested ($n = 22$) ([Image 1](#)). No cases showed high-grade dysplasia.

At the RNA level, the stromal cells in all cases showed upregulated hormone receptors *ER2* (fold change = 29.91, $P = 2.5 \times 10^{-17}$, false discovery rate = 8.8×10^{-16}) and *PR* (fold change = 15.24, $P = 1.68 \times 10^{-14}$, false discovery rate = 3.54×10^{-13}) compared with nonneoplastic liver. An additional ovarian stromal marker, *WT1* (fold change = 19.56, $P = 1.42 \times 10^{-10}$, false discovery rate = 1.55×10^{-9}), was also significantly upregulated [Table 1](#). We validated these findings immunohistochemically on 13 cases of mucinous cystic neoplasm of the liver with available tumor, including the cases examined by RNAseq. All were diffusely positive for estrogen receptor and progesterone receptor in the stroma and focally, strongly positive for WT-1 in the stroma ($n = 13$) [Image 2](#).

At the RNA level and immunohistochemically, we examined the expression of sex cord stromal markers including

Image 1 Histology of mucinous cystic neoplasm of the liver demonstrates occasional luteinized stromal cells (**A**, H&E, $\times 100$; **B-E**, H&E, $\times 200$), ovarian-like stroma, and mucinous epithelium that is cytokeratin 19 positive (**F**, H&E, $\times 100$).

Table 1
Mucinous Cystic Neoplasm of the Liver Immunohistochemical and RNAseq Results

	Immunohistochemistry		RNAseq (n = 6)		
	Positive Staining, % (n)	Staining Intensity	Fold Change	P Value	FDR
Ovarian markers (n = 13)					
Progesterone	100 (13)	Diffuse, Strong	15.24	1.68×10^{-14}	3.54×10^{-13}
Estrogen	100 (13)	Diffuse, Strong	1.29	2.99×10^{-1}	3.95×10^{-1}
WT1	100 (13)	Focal, Strong	19.56	1.42×10^{-10}	1.55×10^{-9}
Sex cord stromal markers (n = 13)					
SF1	100 (13)	Diffuse, Strong	89.9	7.43×10^{-7}	4.32×10^{-6}
Inhibin- α	84 (11)	Focal, strong	9.45	2.38×10^{-11}	2.98×10^{-10}
Melan-A	76 (10)	Focal, strong	Unavailable	Unavailable	Unavailable
Calretinin	84 (11)	Focal, strong	4.51	2.51×10^{-8}	1.90×10^{-7}
Sex hormone biosynthesis pathway (n = 10)					
StAR	NA	NA	99.73	5.96×10^{-10}	5.87×10^{-9}
CSCCE	NA	NA	5.86	3.62×10^{-10}	3.70×10^{-9}
17 β -hydroxysteroid dehydrogenase 1	80 (8)	Focal	10.63	9.01×10^{-17}	2.82×10^{-15}
Aromatase	NA	NA	5.46	1.65×10^{-2}	3.35×10^{-2}
17 α -hydroxylase / 17-20 lyase	NA	NA	2.51	1.24×10^{-1}	1.91×10^{-1}
3 β -hydroxysteroid dehydrogenase	NA	NA	0.32	6.07×10^{-3}	1.41×10^{-2}

CSCCE, cholesterol side chain cleavage enzyme or cholesterol desmolase; NA, not assessed; RNAseq, RNA sequencing; StAR, steroidogenic acute regulatory protein.

SF-1, *melan-A*, *inhibin- α* , and *calretinin*.²⁶ The stromal cells demonstrated upregulation of all the sex cord stromal markers at the RNA level compared with nonneoplastic liver: *SF-1* (fold change = 89.88, $P = 7.43 \times 10^{-7}$, false discovery rate = 4.32×10^{-6}), *inhibin- α* (fold change = 9.45, $P = 2.38 \times 10^{-11}$, false discovery rate = 2.98×10^{-10}), and *calretinin* (fold change = 22, $P = 2.51 \times 10^{-8}$, false discovery rate = 1.9×10^{-7}). The *MLANA* transcript (which encodes melan-A) was not captured in our RNAseq experiment. SF-1 immunoexpression was strong and diffusely positive in the stroma of all mucinous cystic neoplasm of the liver cases tested (n = 13), whereas calretinin, inhibin- α , and melan-A were expressed focally strong in 84% (11/13), 84% (11/13), and 76% (10/13) of the mucinous cystic neoplasm of the liver cases, respectively **Image 3**.

Stroma of Mucinous Cystic Neoplasm of the Liver Demonstrates Overexpression of Steroidogenic Markers and Enzymes Involved in Estrogen Biosynthesis

At the RNA level, we looked for evidence of steroidogenesis and estrogen synthesis, assessing the following enzymes involved in sex hormone biosynthesis (the enzymes and their fold change, P value, and false discovery rate, respectively, are listed): aromatase (*CYP19A1*; 5.46, 1.65×10^{-2} , 3.35×10^{-2}), 17 α -hydroxylase/17,20 lyase/17,20 desmolase (*CYP17A1*; 2.51, 1.24×10^{-1} , 1.91×10^{-1}), 17 β -hydroxysteroid dehydrogenase 1 (*HSD17B1*; 10.63, 9.01×10^{-17} , 2.82×10^{-15}), steroidogenic acute regulatory protein (*StAR*; 99.73, 5.96×10^{-10} , 5.87×10^{-9}), and cholesterol side chain cleavage enzyme or cholesterol desmolase (*CYP11A1*; 5.86, 3.62×10^{-10} , 3.70×10^{-9}) (Table 1).

We confirmed the expression of 17 β -hydroxysteroid dehydrogenase 1 at the protein level using immunohistochemistry because it is the final step in production of estradiol. Stromal cells were focally positive in 8 of 10 cases with available material **Image 4**.

These data indicate that the sex hormone biosynthesis pathway is active in mucinous cystic neoplasm of the liver with significant upregulation of steroidogenic enzymes **Figure 1**. Overall, the combination of transcriptomic and immunohistochemical results suggests the stroma of mucinous cystic neoplasm of the liver is potentially both hormonally responsive and capable of producing sex hormones.

Differential Gene Expression and Pathway Analysis Indicates Upregulation of Hedgehog and Wnt Pathways

Ingenuity pathway analysis (Qiagen) was utilized to evaluate molecular pathway dysregulation. Gene expression pathway analysis revealed significant activation of 2 oncogenic pathways: the hedgehog pathway (z score = 2.53, $P = 9.19 \times 10^{-4}$) and Wnt pathway (z score = 1.00, $P = .0068$). Hedgehog pathway upregulation was validated at the protein level with GLI1 immunohistochemistry. All 13 cases showed strong and diffuse GLI1 expression in both the stromal and epithelial cells **Image 5**.

Gene expression analysis also identified significant downregulation of the T-helper 1 pathway (z score = -3.04, $P = 5 \times 10^{-18}$) and the T-helper 2 pathway (z score = -5.42, $P = 2 \times 10^{-18}$). Furthermore, a marker of immune response IFN- γ (*IFNGR1*; fold change = -2.23, $P = 1.47 \times 10^{-5}$, false discovery rate = 6.51×10^{-5}) was

Image 2 In the ovarian-type stroma of mucinous cystic neoplasm of the liver (**A**, H&E, $\times 40$), immunohistochemical markers demonstrate strong and diffuse expression of estrogen receptor (**B**, $\times 40$) and progesterone receptor (**C**, $\times 40$) and strong focal WT-1 (**D**, $\times 100$).

downregulated. The downregulation of the T-helper 1 and 2 pathways and IFN- γ suggests that the tumor microenvironment is immune suppressed.

DNA Sequencing Analysis

Targeted next-generation sequencing analysis did not reveal any recurrent somatic mutations. Specifically, *RNF43*, *KRAS*, and *GNAS* were wild type in all cases tested ($n = 22$). We identified heterozygous somatic single-nucleotide variants in *SMO* in 2 separate cases that were predicted to be pathogenic in 2 independent software programs, SIFT and Mutation Taster. The *SMO* mutations were c.608C>A (allele frequency 50%) and

c.910G>A (allele frequency 27%). The *SMO* gene is part of the hedgehog signaling pathway.

Discussion

Mucinous cystic neoplasm of the liver is characterized by a mucinous, biliary-type, or mixed mucinous–biliary-type epithelium with adjacent ovarian-type stroma. This study is the first molecular investigation of mucinous cystic neoplasm of the liver. We identified several novel observations.

Our study shows that the stroma of mucinous cystic neoplasm of the liver is not only histologically similar to that of the ovary but shows features suggestive of hormone

Image 3 In stroma of all mucinous cystic neoplasm of the liver cases (**A**, H&E, $\times 40$), immunohistochemical markers demonstrate strong and diffuse SF-1 expression (**B**, $\times 40$), whereas melan-A (**C**, $\times 40$), inhibin- α (**D**, $\times 40$), and calretinin (**E**, $\times 100$) were focally strongly positive in a subset of cases.

Image 4 Immunohistochemical expression of 17 β -hydroxysteroid dehydrogenase 1 demonstrates strong focal positivity in the stroma cells of mucinous cystic neoplasm of the liver (**A**, $\times 100$; **B**, $\times 200$).

Figure 1 Diagram of sex hormone biosynthesis showing enzymes interrogated by RNA sequencing that are significantly upregulated and downregulated. ^a17 α -hydroxylase and C17-20 lyase are upregulated but not statistically significant. 3 β -HSD, 3 β -hydroxysteroid dehydrogenase; 17 β -HSD, 17 β -hydroxysteroid dehydrogenase 1.

responsiveness and hormone production at RNA and protein levels. Taken together with the finding of FOXL2 protein expression, these data provide the first molecular-level evidence that suggests mucinous cystic neoplasm of the liver arises from ectopic primordial cells of the gonad or from a cell type in the liver that has the capacity to show full ovarian cortical cell differentiation. Specifically, morphologically, we identified morphological stromal changes akin to those seen in the normal ovary, expression of hormone receptors (estrogen receptor and progesterone receptor), and evidence of expression of steroidogenic

enzymes (StAR, CYP17, CYP19 and 17 β -hydroxysteroid dehydrogenase 1). These data are consistent with observations in an immunohistochemical study by Kumata et al,¹¹ who noted expression of steroid markers in mucinous cystic neoplasms of the liver and pancreas including StAR, CYP17, CYP19, 17 β -hydroxysteroid dehydrogenase 1, and CYP11. Our study adds to theirs by providing molecular confirmation along with identification of other markers expressed in normal ovarian cortex. The pattern of elevation of steroidogenic enzyme transcripts in our study is consistent with estradiol production,

Image 5 Immunohistochemical expression of GLI1 in the ovarian-like stroma and epithelium of mucinous cystic neoplasm of the liver demonstrates strong and diffuse positivity (**A**, $\times 100$; **B**, $\times 200$).

although we were not able to specifically test for estradiol in the formalin-fixed paraffin-embedded tissues. These findings have a number of implications. The first is diagnostic; SF-1, in particular, and other stromal markers are expressed in eutopic or ectopic ovaries or adrenal glands in routine clinical practice. The data from our study confirm that mucinous cystic neoplasms of the liver consistently display expression of SF-1. Consequently, SF-1 expression from the stroma of an abdominal or retroperitoneal mass does not confirm an ovarian or adrenocortical primary. The second implication is that there is a key role for estrogen in the pathogenesis of biliary neoplasia. This notion is independently supported by studies that have shown estrogen stimulates proliferation of cholangiocarcinoma, is involved in the biology of the inflammatory microenvironment of neoplastic biliary epithelium and the finding of increased serum estrogen in male patients with cholangiocarcinoma, and correlates with proliferation and invasiveness.²⁷⁻³⁰

Our findings agree with a recent hypothesis regarding the histogenesis of mucinous cystic neoplasm of the liver. During embryogenesis, primordial germ cells that give rise to embryonic gonads begin descent at the third week of development; by the fifth week, they are at the dorsal mesentery, which gives rise to the body and tail of the pancreas. This process has been proposed by investigators as a potential origin for mucinous cystic neoplasm of the pancreas.³¹ Primordial germ cells do not migrate through the liver. However, the liver is in very close proximity to the dorsal mesentery during the fifth week of embryonic life, and cells from the dorsal mesentery may enter the ventral mesentery where the liver is

developing. Not only is there a close physical relationship between the 2 organs but there is a close temporal relationship in developmental processes as the biliary duct and pancreatic duct systems connect to drain in the duodenum. In addition, the 2 organs are under the developmental control of a common transcription factor during this time period. The transcription factor *GATA4*, which is responsible for the development of the gonadal ridge where the gonads will develop, is responsible for the growth of the liver and pancreas during this time.³² Thus, aberrant descent of primordial germ cells may be the key event responsible for the histogenesis of mucinous cystic neoplasm of the liver, but this requires additional study.

A related hypothesis is that the ovarian-like stroma may be a recapitulation of the periductal fetal mesenchyme that forms a cuff around the fetal pancreatic and hepatic ducts in a manner and distribution very similar to the way ovarian-like stroma follows the epithelium in mucinous cystic neoplasms.^{33,34} It is possible that this distinctive fetal mesenchyme may include embryonic cells with a capacity to transdifferentiate toward ovarian stromal cells under the right conditions. This transdifferentiation may be so complete that it shows all characteristics of ovarian cortex including luteal type cells.

Our study shows some similarities between mucinous cystic neoplasm of the liver and mucinous cystic neoplasm of the pancreas, as described previously, and also some differences comparing our results with other results from prior studies. We have already described the possible potential similar embryonic origin. In addition, the similarity of the stromal appearance raises the possibility that mucinous

cystic neoplasm of the pancreas may also be associated with steroid hormone production. In addition, the expression analysis of mucinous cystic neoplasm of the liver identified Wnt pathway activation but without any mutations in Wnt pathway members, similar to what has been identified in mucinous cystic neoplasm of the pancreas.^{13,15,16,35} Interestingly, the results of DNA sequencing provide differences such as no identification of *KRAS* or *RNF43* mutations in any of our cases, raising the possibility that the molecular biology of the pancreatic counterpart is dissimilar at some levels.^{13,16,17,19,20} Along these lines, a study by Fujikura et al²⁰ demonstrated *KRAS* mutations in 4 of 17 pancreatic mucinous cystic neoplasms including cases with both high- and low-grade dysplasia, whereas only 1 of 8 hepatic mucinous cystic neoplasms had *KRAS* mutations, and that case showed high-grade dysplasia. These data combined with ours from a series of cases without high-grade dysplasia suggest that *KRAS* is not a major driver of the early development of mucinous cystic neoplasm.

Our findings may also have implications for mixed epithelial stromal tumor of the kidney, which shares some histomorphology with mucinous cystic neoplasm of the liver.^{34,36} However, detailed examination is beyond the scope of this study. Nevertheless, it is possible that ovarian-type stroma supports tumor development in mixed epithelial stromal tumor of the kidney.

Two pathways that emerged as significantly activated in mucinous cystic neoplasm of the liver were the hedgehog and Wnt pathways; the latter was mentioned earlier. These RNA-level data accord with the expression of *GLI1* by the stromal and epithelial cells, as seen by immunohistochemistry using the same antibody and conditions that were used for prior work on another hedgehog-driven tumor, gastroblastoma.³⁷ *GLI1* is a key target gene for the hedgehog pathway. The Gorlin syndrome, characterized by germline *PTCH1* mutations, is a model for examining aberrant hedgehog signaling because loss of *PTCH1* corresponds to unrestrained activation of the hedgehog pathway. Interestingly, in the Gorlin syndrome, there are rare reports of mesenchymal liver tumors.^{38,39} Although no mucinous cystic neoplasms of the liver have been reported in patients with the Gorlin syndrome, the noted findings provide additional evidence that aberrations of the hedgehog pathway may contribute to development of mesenchymal tumors in the liver. Further support for the importance of the hedgehog pathway in our study was obtained at the DNA level. *SMO*, a gene in the hedgehog pathway, showed heterozygous mutations in 2 cases. Deleterious mutations in *SMO* may contribute to hedgehog pathway activation. The possibility of large insertion deletions in *SMO* or other genes in the hedgehog pathway could not be examined with our

DNA sequencing assay. Taken together, the findings of our study suggest that mucinous cystic neoplasm of the liver is driven by hedgehog pathway abnormalities.

In terms of significantly downregulated pathways, we noted downregulations of T-helper 1 and 2 pathways in mucinous cystic neoplasm. This finding also corresponded to RNA-level downregulation of interferon γ . This interesting observation logically supports how the neoplastic cells could escape immune surveillance and proliferate. The immunosuppressive nature of ovarian stroma is the subject of ongoing investigation.^{40,41}

In summary, our data demonstrate that mucinous cystic neoplasm of the liver is characterized by a stroma that not only morphologically resembles ovarian stroma but shows, at the protein and RNA levels, characteristics of normal ovarian stroma including both features suggesting hormonal responsiveness and hormone production. This finding has implications for our understanding of the biology of biliary neoplasia. Our data also show that mucinous cystic neoplasm of the liver is a tumor likely driven by the hedgehog pathway and is characterized by an immunosuppressed microenvironment.

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