

2013 Colorado Society of Pathology

Immunohistochemistry for hepatocellular carcinoma

Sanjay Kakar, MD
University of California, San Francisco

Hepatocellular carcinoma

Immunohistochemistry

- Commonly used markers: strengths and limitations
- Different clinical scenarios

Distinction from

- Dysplastic nodule
- Hepatocellular adenoma

HCC immunohistochemistry

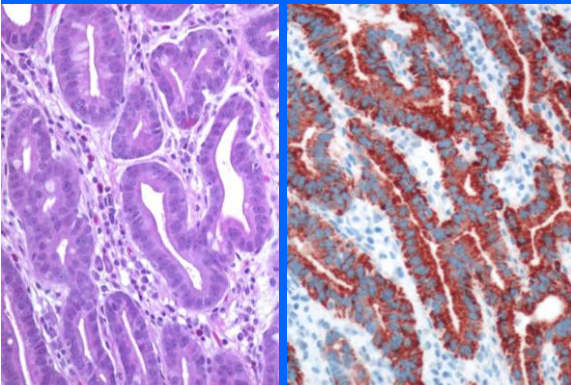
Hepatocellular differentiation

- Hep Par 1
- Polyclonal CEA
- Glypican-3
- Arginase-1
- Others: AFP, CD10, villin, TTF-1

Hep Par 1

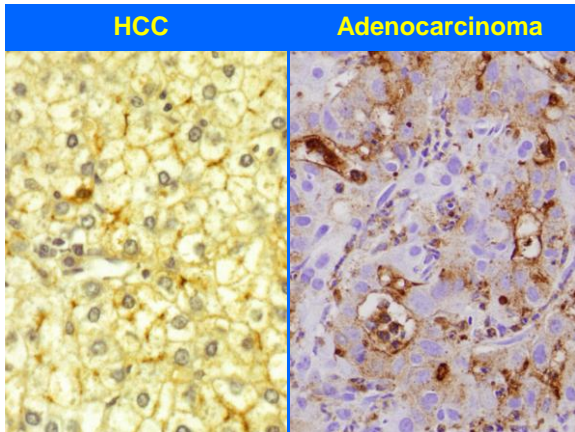
Strengths	Limitations
High sensitivity and specificity (>80%)	Negative: 50% of poorly differentiated, scirrhous HCC
Most adenocarcinomas are negative	Focal staining 10-20%
Other polygonal cell tumors often negative	Positive: 20-30% lung, esophageal, gastric adenoCA
Well studied in different tumors	

Hep Par 1 in gastric adenocarcinoma



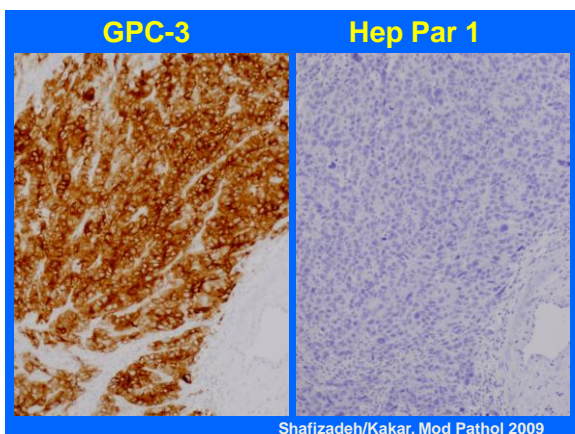
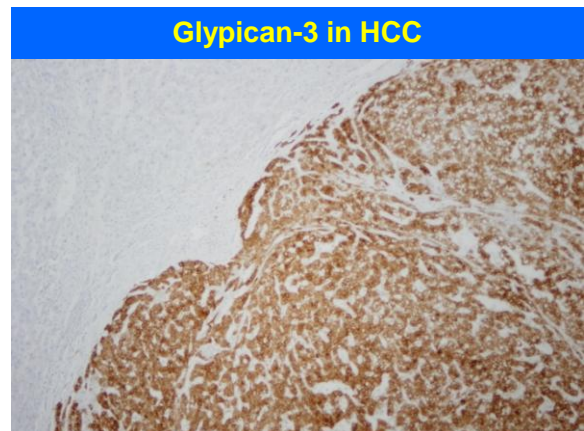
Polyclonal CEA

Strengths	Limitations
High sensitivity (>80%)	Negative: 50% of poorly differentiated, scirrhous HCC
Canalicular pattern is specific	Can be difficult to interpret due to cytoplasmic staining

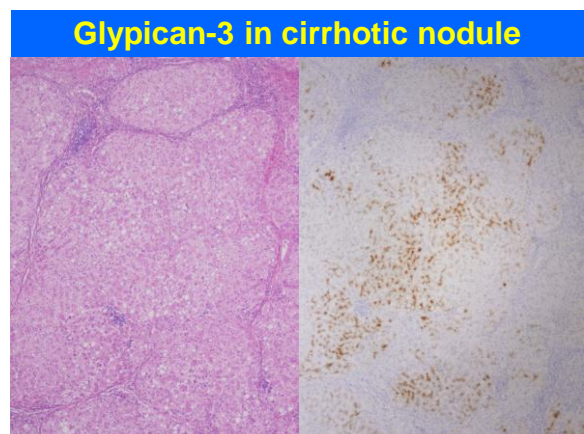


Glypican-3	
Strengths	Limitations
High sensitivity in poorly differentiated, scirrhous HCC (>80%)	Low sensitivity in well (<50%) and moderately differentiated HCC
Negative in adenoma and most high-grade dysplastic nodules	Positive in occasional cirrhotic nodules
	Positive in other tumors: yolk sac, melanoma, some adenocarcinomas

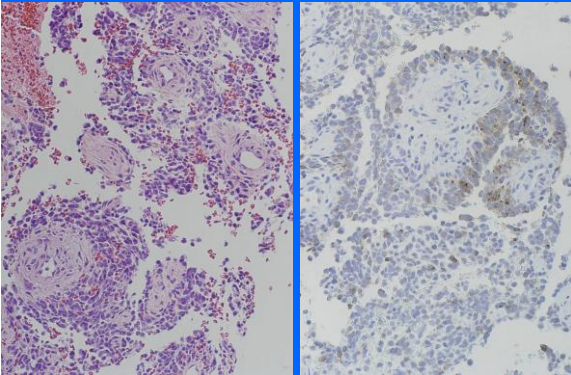
Glypican 3 Expression in Human Nonneoplastic, Preneoplastic, and Neoplastic Tissues	
A Tissue Microarray Analysis of 4,387 Tissue Samples Baumhoer, Am J Clin Pathol 2008	
Hepatocellular carcinoma	29 (66)
Squamous cell carcinoma of the lung	27 (54)
Liposarcoma	15 (52)
Testicular nonseminomatous germ cell tumor	32 (52)
Cervical intraepithelial neoplasia (grade 3)	12 (41)
Malignant melanoma	14 (29)
Adenoma of the adrenal gland	4 (27)
Schwannoma	12 (26)
Malignant fibrous histiocytoma	7 (24)
Adenocarcinoma of the stomach (intestinal subtype)	9 (20)
Chromophobe renal cell carcinoma	3 (20)
Invasive lobular carcinoma of the breast	9 (20)
Medullary carcinoma of the breast	5 (17)
Squamous cell carcinoma of the larynx	8 (16)
Small cell carcinoma of the lung	8 (16)
Invasive transitional cell carcinoma of the urinary bladder	7 (16)
Mucinous carcinoma of the breast	4 (15)
Squamous cell carcinoma of the cervix	6 (15)



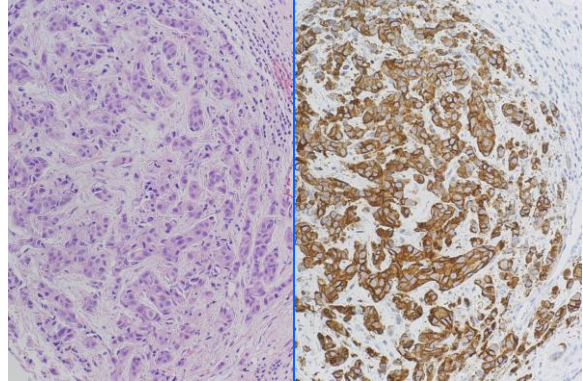
Shafizadeh/Kakar, Mod Pathol 2009



Glypican-3 in melanoma



Glypican-3 in metastatic breast CA

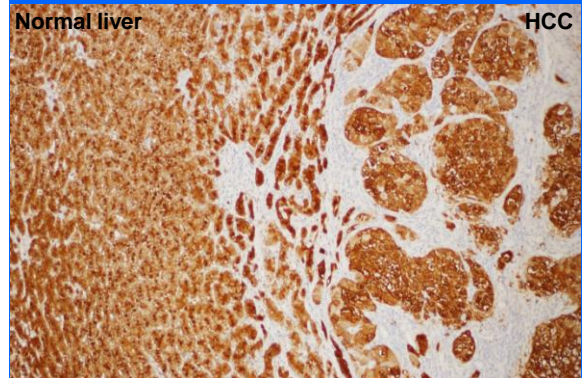


Arginase-1

Strengths	Limitations
High sensitivity (90%), including poorly differentiated, scirrhous HCC	Limited experience
High specificity (>90%): most other tumors are negative	Rare positive staining in other tumors: -Prostatic adenocarcinoma -Cholangiocarcinoma (weak, focal)

Tan, AJSP, 2010
Philips/Kakar, USCAP 2012

Arginase-1: nuclear and/or cytoplasmic



Hepatocellular markers

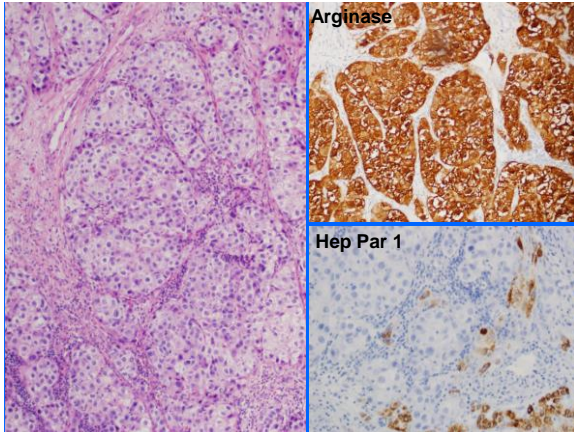
	Well-diff	Mod diff	Poorly diff
Hep Par 1	100%	83%	46%
Arginase-1	100%	96%	85%

Tan, AJSP, 2010

Sensitivity of commonly used hepatocellular markers

	Well diff	Mod diff	Poorly diff
Hep Par 1	100%	98%	63%
pCEA	92%	88%	60%
GPC-3	62%	83%	86%
Arginase-1	100%	100%	97%

Philips/Torbenson/Kakar, USCAP 2012



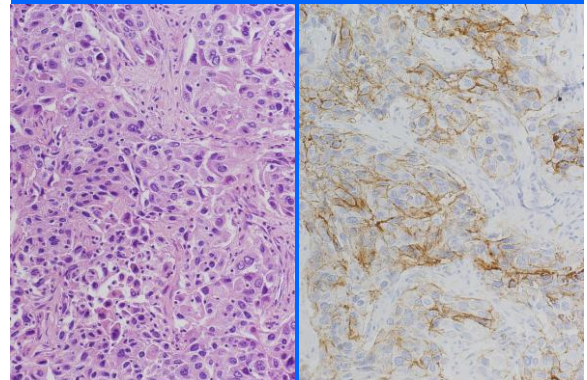
Other markers

Marker	Limitations
AFP	Low sensitivity (30%), background staining
Villin, CD10	Similar to polyclonal CEA
TTF-1	Staining similar to Hep Par 1 Clone-dependent
CD34	Sinusoidal pattern not specific
Albumin in situ hybridization	Not widely available

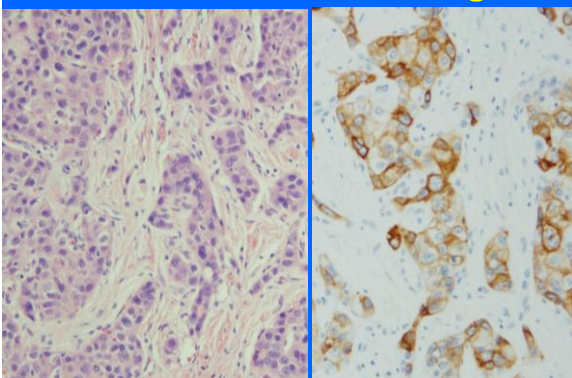
'Adenocarcinoma' markers

Marker	Use
MOC31 (EPCAM)	Most adenocarcinomas
CK7	Neuroendocrine tumors
CK19	HCC: 5-20%
Pan CK (AE1/AE3)	Positive in most HCCs
Site specific markers	TTF-1, napsin A, PSA, ER/PR, GCDPF-15, CDX-2

HCC with MOC31 staining



HCC with CK19 staining



HCC: immunohistochemistry

Non-cirrhotic liver	Cirrhotic liver
Benign: HCA, FNH	High-grade dysplastic nodule
Cholangiocarcinoma Metastatic adenocarcinoma	Cholangiocarcinoma
Polygonal cell tumors: NET, adrenocortical carcinoma, RCC, melanoma, sarcomas	Metastatic tumors: rare

Needle biopsy for HCC

No stains necessary

- Bile production
- Cirrhotic liver: characteristic features
 - Trabecular pattern
 - Fat and/or Mallory hyaline

'Mesothelioma' approach

2 hepatocellular markers	2 'adenocarcinoma' markers
Arginase-1 Glypican-3 Hep Par 1 Polyclonal CEA	MOC31 CK19 CK7
Other markers	Clinical setting
TTF-1, CDX-2, ER/PR etc	If appropriate
2 marker approach Arg-1, MOC31	Limited material

Four groups

	Arginase-1	MOC31
Group 1	+	-
Group 2	-	+
Group 3	+	+
Group 4	-	-

Arginase+ MOC31 –

- Establishes the diagnosis of HCC in most situations
- Additional work-up if
 - clinical info/morphology not typical
 - staining pattern focal

Arginase – MOC31 +

Differential diagnosis

- Adenocarcinoma
- Polygonal cell tumors:
 - RCC, NE tumor
- HCC (rare)

Arginase+ MOC31+

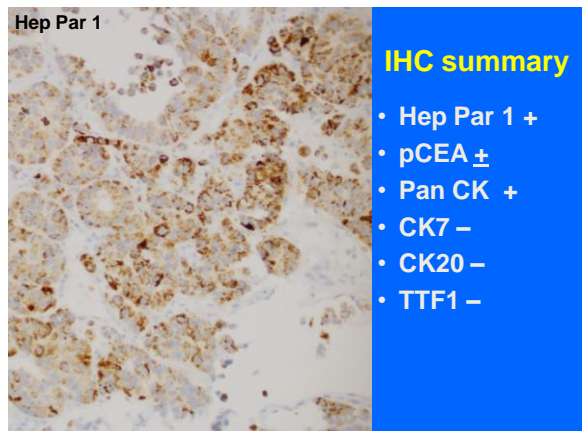
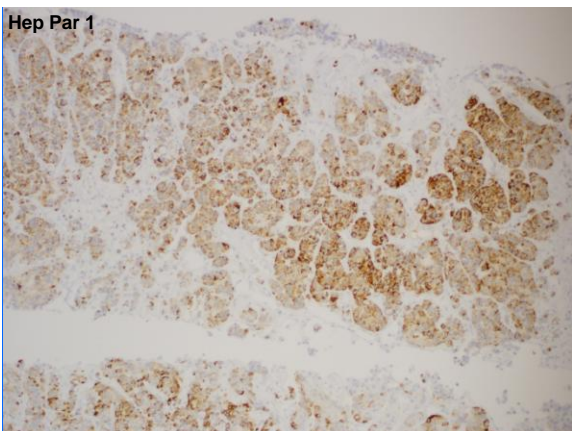
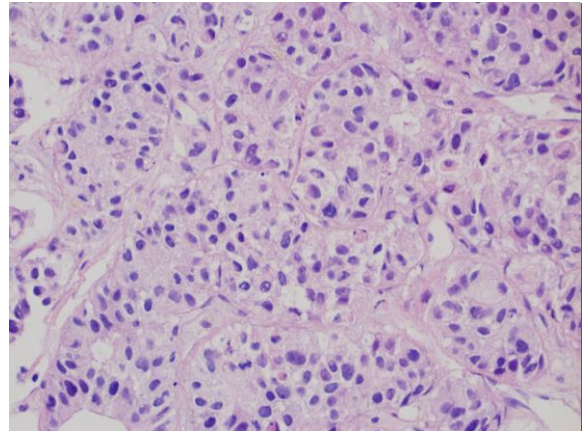
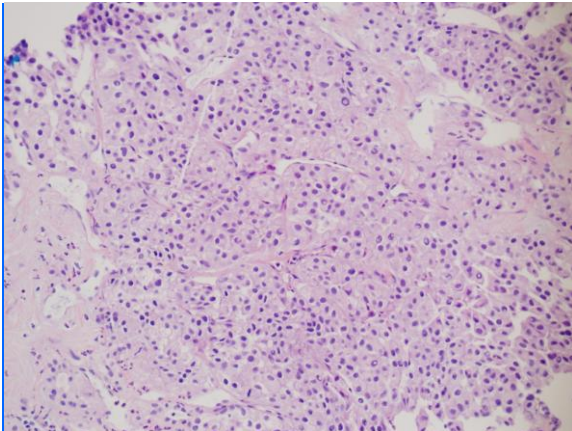
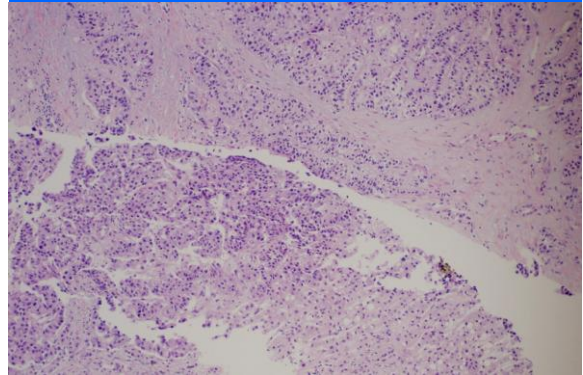
Differential diagnosis

- MOC31+ HCC
- Adenocarcinoma/NET with arginase expression (rare)

Arginase – MOC31 –

Pancytokeratin +	Pancytokeratin -
HCC	Melanoma
Adenocarcinoma	Adrenocortical CA
NE tumors, RCC	Angiomyolipoma
Urothelial CA	Sarcomas with epithelioid pattern
Squamous cell CA	

Case 1: 66/M, 6 cm liver mass
no other known tumor



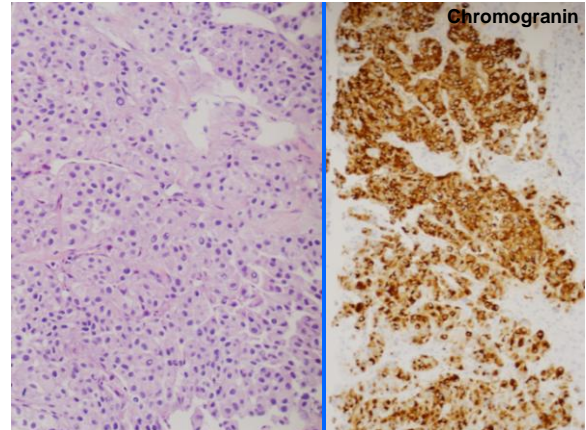
IHC summary

- Hep Par 1 +
- pCEA ±
- Pan CK +
- CK7 -
- CK20 -
- TTF1 -

Additional stains

Hep Par	CK7	Arginase-1	MOC31
+	-	-	+

- HCC (rare)
- Non-HCC with aberrant Hep Par
Adenocarcinoma
NET
Renal cell carcinoma



Chromogranin

	HCC	NET
Arg-1, GPC-3, Hep Par 1	Positive	Hep, GPC-3 rarely positive
MOC31 CK19, CK7	5-20%	Usually positive
Chromogranin Synaptophysin CD56	Negative Rare positive Variable	Positive Positive Positive

Immunostaining in HCC and NET

	Hep Par 1	GPC-3	Synapto- physin	Chromo- granin A	CD56
HCC (n = 114)	92	67	3	0	7
NET (n = 48)	0	0	47	40	39

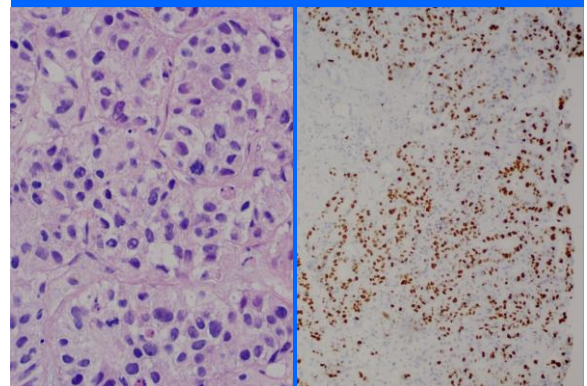
Zhou/Frankel, USCAP meeting, 2011

WHO terminology

Term	Mitoses/10HPF	Ki67 index
Neuroendocrine tumor, grade 1	<2	≤2%
Neuroendocrine tumor, grade 2	2-20	3-20%
Neuroendocrine carcinoma, large cell or small cell	>20	>20%

Mitoses: 13/10 hpf

Ki-67 index 50%



Diagnosis

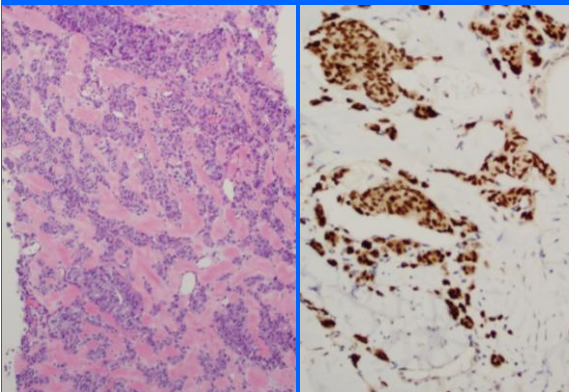
Large cell neuroendocrine carcinoma (grade 3, WHO 2010 grading scheme)

Primary site of NET

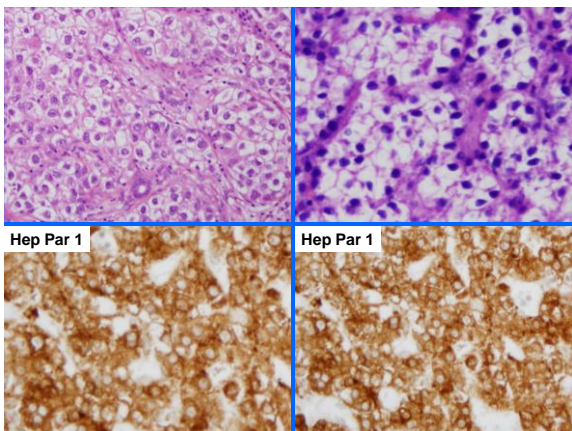
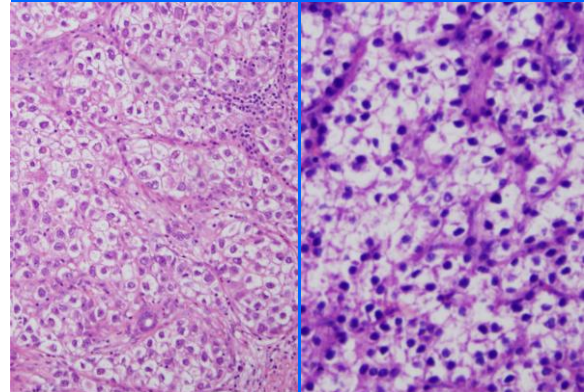
Immunohistochemistry	Primary site	Comments
TTF-1	Lung	Not specific; NETs at other sites can be TTF-1 positive
CDX-2	Intestine	Occasional positivity at other sites: pancreatic NET
PAX-8	Pancreas	Limited data

Pancreatic NET

PAX8

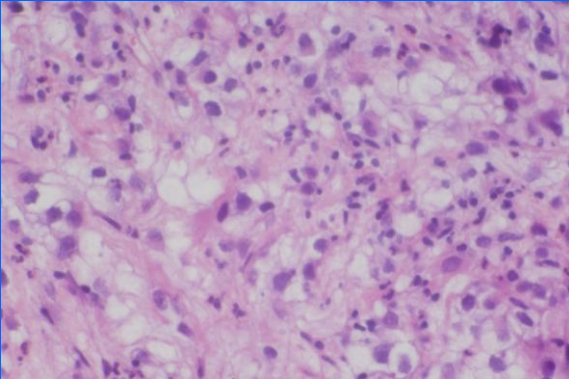


HCC or renal cell carcinoma



Marker	HCC	Clear cell RCC
Arg-1, GPC-3	Positive	Negative
Hep Par 1	Positive	Negative
PAX-2 or PAX-8	Negative	Positive Other GU/GYN tumors
RCC marker, EMA, vimentin	Negative	Positive
CD10	Canalicular	Membranous

Two-stain approach for clear cell tumors:
Arg-1 and PAX-2/PAX-8

Case 2: 60M, multiple liver and lung masses**Initial work-up**Negative

Hep Par 1

pCEA

CK7

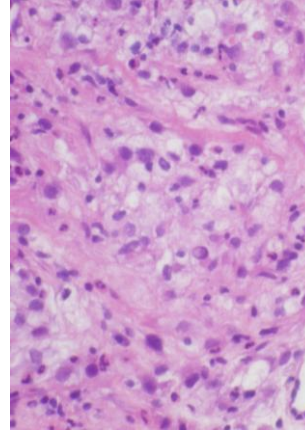
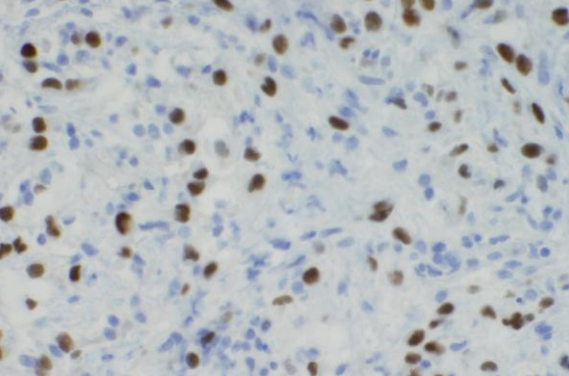
TTF-1

PSA

RCC marker

Possible kidney mass

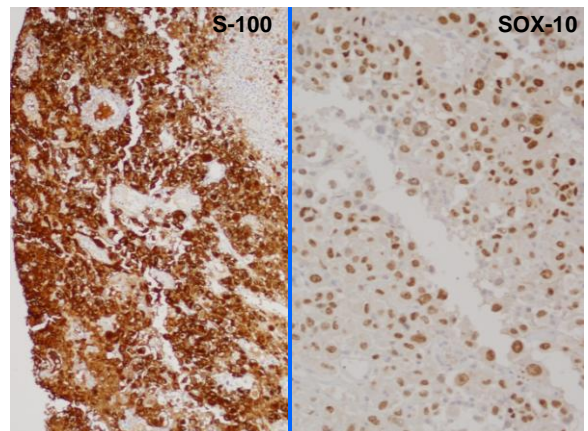
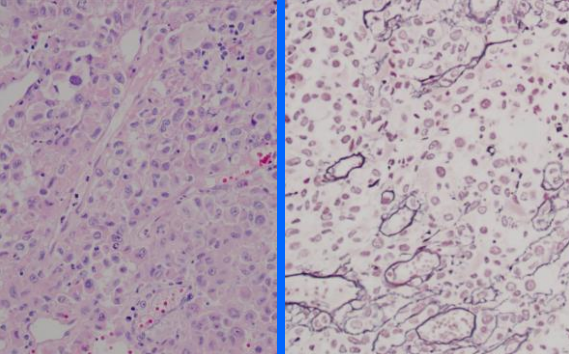
No tissue in the block

**PAX-2 nuclear+: metastatic RCC****HCC vs. polygonal cell tumors**

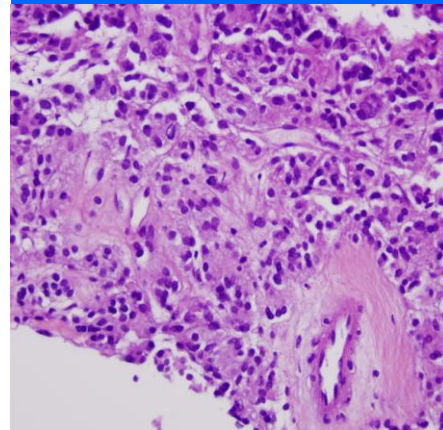
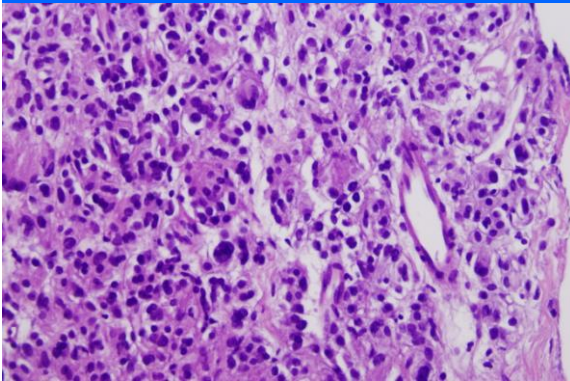
Polygonal cell tumor	Marker
Adrenocortical CA	Inhibin Melan A
Epithelioid angiomyolipoma	SMA HMB-45, Melan A
Melanoma	SOX10, S-100 HMB-45, Melan A

Arginase, Hep Par 1: negative

GPC-3: melanoma

Case 3: 72/M, tumor nodules in liver, lung, bones, primary unknown

Case 4: 50F, no cirrhosis, 5 cm liver tumor



Negative

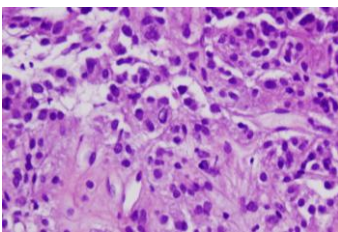
Hep Par 1
pCEA
CK7
CK20

Differential diagnosis

- Hep Par 1 negative HCC
- CK7 negative adenocarcinoma
- Polygonal cell tumor:
 - Neuroendocrine tumor
 - Renal cell carcinoma
 - Adrenocortical carcinoma
 - Melanoma
 - Angiomyolipoma

Hepatic angiomyolipoma *

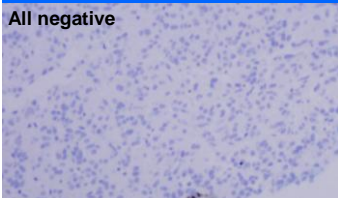
- Monotypic: lacks 'angio' and 'lipoma' components
- Myo component is often epithelioid
- Not associated with TS
- IHC: Hep Par 1, MOC31, CK: -ve
Smooth muscle, HMB 45: +ve



Round 2

- Arg, GPC-3
- MOC31, CK19
-
- CG, Syn
- SOX-10, S-100
- PAX2
- Inhibin
- SMA, HMB-45
- Pan CK

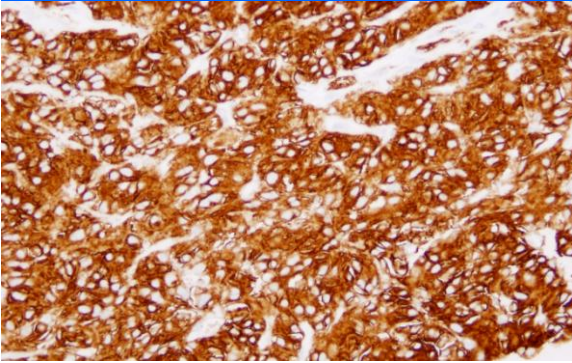
All negative



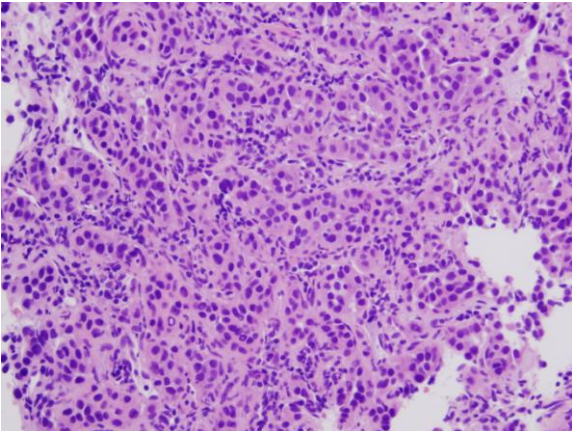
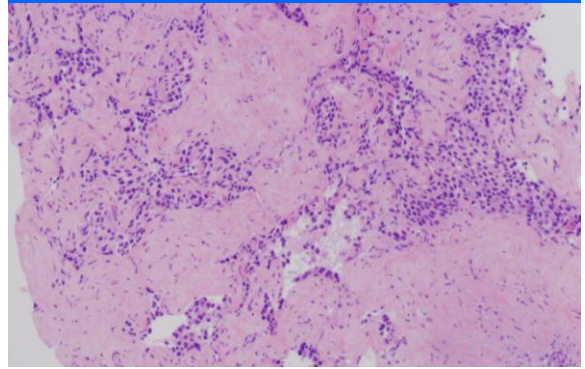
Arginase – CK19 –

Pan CK +	Pan CK -
HCC	Melanoma
Adenocarcinoma	Adrenocortical CA
NE tumors, RCC	Angiomyolipoma
Urothelial CA	Sarcomas with
Squamous cell CA	epithelioid pattern

Kit: Metastatic GIST

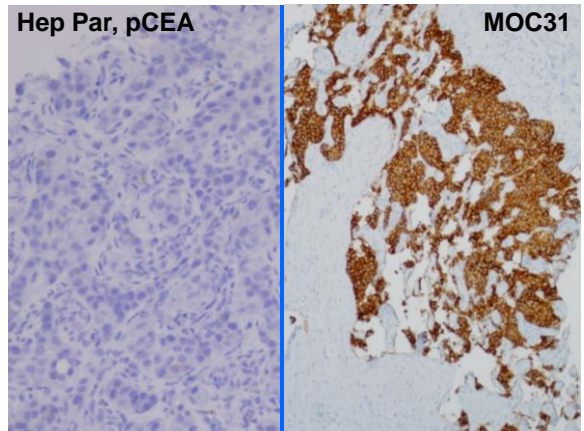


Case 5: 55/M with cirrhosis, 6 cm liver mass



Hep Par, pCEA

MOC31

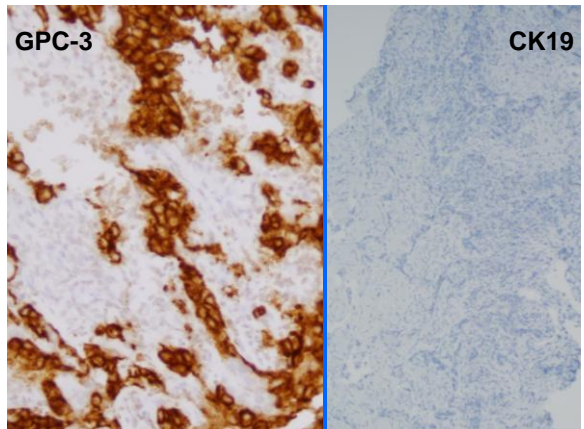


Misleading features

- Abundant stroma
- Immunophenotypic features
 Negative: Hep Par 1, pCEA
 Positive: MOC31

GPC-3

CK19



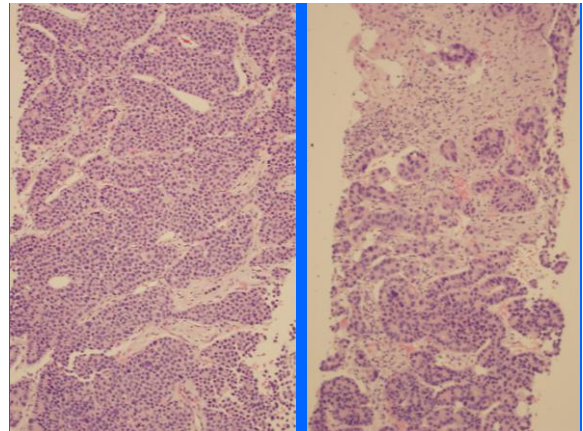
Clinicopathologic features	Scirrhou HCC (>50% fibrous stroma)	Conventional HCC *
Multinodular	65%	19%
Fibrous capsule	0	71%
Portal tracts	70%	16%
Necrosis	0	70%
Imaging		
Peripheral enhancement	62%	3%
Prolonged enhancement	95%	4%
Areas of venous washout	19%	99%
No cirrhosis	15-25%	15-20%
Outcome	Better/same/worse	

Immunostain	Scirrhou HCC	Conventional HCC *
Hep Par 1	17-20%	80-90%
pCEA	33%	60-80%
K7	58-65%	0-20%
K19	50%	0-10%
MOC31	64%	5-11%
Arginase	95%	95%
Glypican-3	95%	70-80%

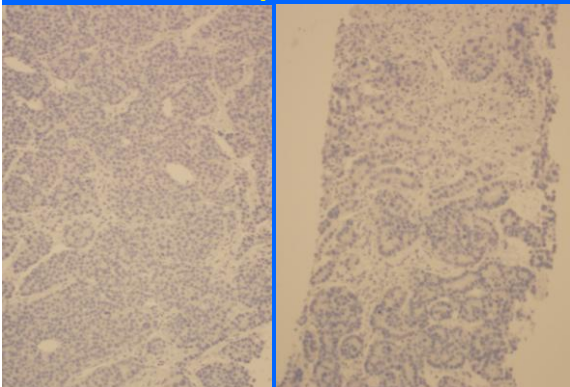
Matsuura, Histopath, 2005
Kings/Kakar, Mod Pathol, 2013

Case 7 *

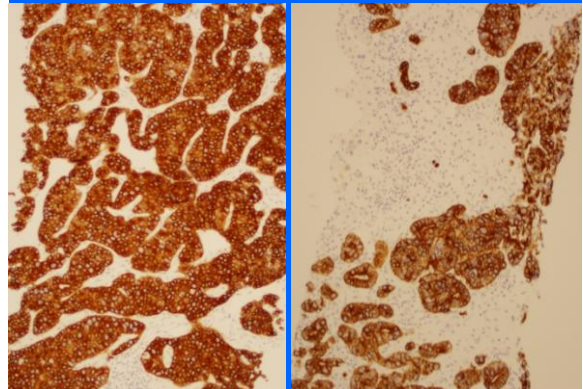
- 62 year old woman with a 6 cm liver mass
- No clinical evidence of chronic liver disease



Hep Par 1



CK19



Differential diagnosis

- Hepatocellular carcinoma with pseudoglandular differentiation
- Cholangiocarcinoma with poorly differentiated component
- Combined hepatocellular-cholangiocarcinoma

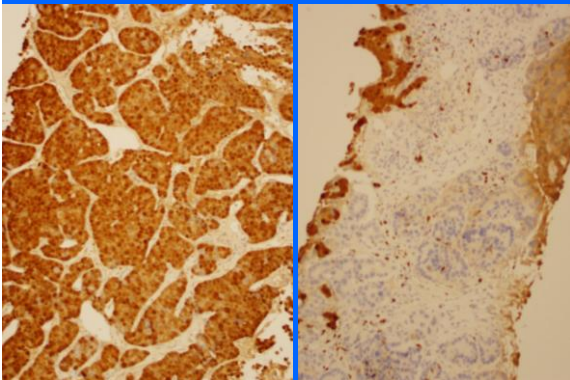
HCC or CC: clinical impact

HCC	CC or Combined HCC-CC
Lymph nodes may not be removed	Lymph node dissection is routine

HCC	CC or Combined HCC-CC
Sorafenib, transarterial chemoembolization	Gemcitabine-based or fluoropyrimidine-based

HCC	CC or Combined HCC-CC
Liver transplant: Milan/UCSF criteria	Likely denial

Arginase-1



CK19+ HCC

stem cell phenotype

- Microvascular invasion
- Fibrous stroma
- Independent predictor of poor survival

Kim, Hepatology, 2011

HCC to CC spectrum: a new classification?

HCC	CK19-
HCC	CK19+
Scirrhous HCC	CK19±
HCC-CC, classical	CK19+
CC	CK19+

Evidence for CC component

Morphology	Discrete gland formation Mucin: positive Fibrous stroma
IHC	CK19, CK7, MOC31: strong + Arg: negative GPC-3: negative (>90%)

HCC subtypes

HCC subtype	Unusual features
Scirrhous HCC	Hep, pCEA: often negative Arg-1, GPC: reliable MOC31, CK19: can be +
Fibrolamellar carcinoma	CK7 usually positive Rare: mucin + NE markers + CD68+ may be helpful
Combined HCC-CC	CK19+ alone is not enough for diagnosis of CC component Distinct glands, mucin for CC

Summary

Setting	Approach
Bile, typical morphology in cirrhosis	No stains
Limited biopsy or Cirrhosis, not typical	2 stain approach: Arginase-1, MOC31
Most situations	4 stain approach: Arginase-1, GPC-3/Hep Par 1 MOC31, CK19

- Avoid large reflex staining panels
- Avoid less useful markers like AFP
- Use site-specific markers judiciously

HCC vs. high grade dysplastic nodule

Terminology of HCC

Small HCC: <2 cm

High likelihood of cure

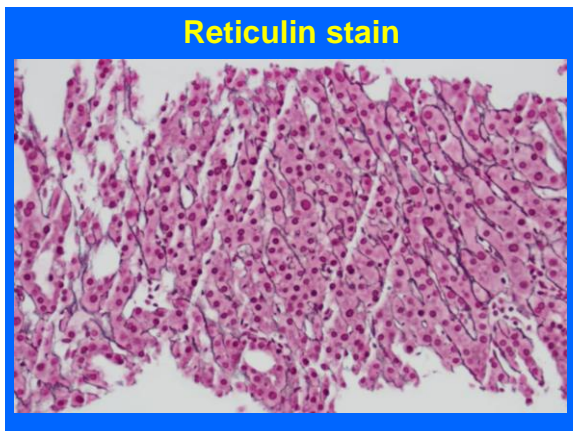
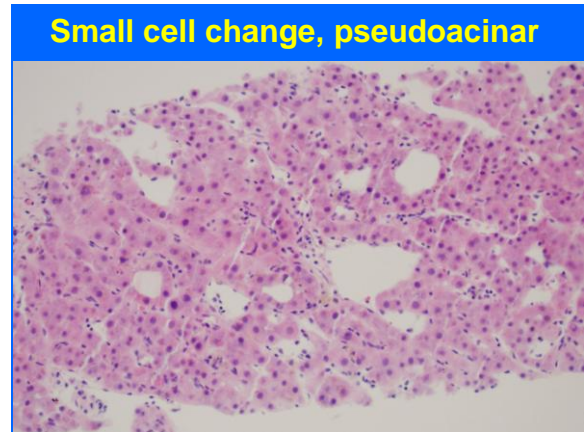
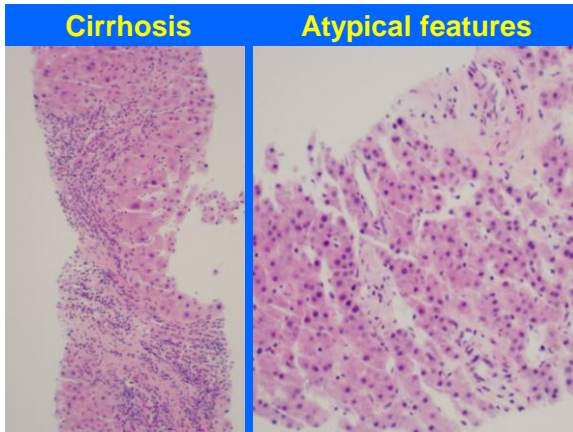
- Progressed HCC: Typical features
Nodular HCC
- Early HCC: Resemble HGDN
Vaguely nodular HCC
Stromal invasion

HGDN vs HCC: clinical implications

High-grade dysplastic nodule	Rebiopsy ?Ablation
Hepatocellular carcinoma	Ablation or resection Priority for transplant

Case 8

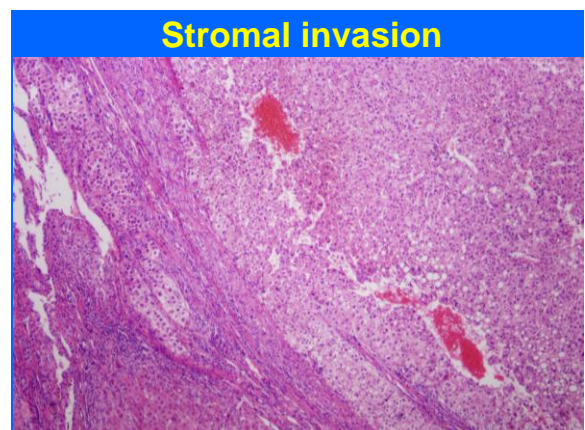
- 48 year old male with chronic hepatitis C and cirrhosis
- 2.5 cm hepatic mass noticed on screening ultrasound



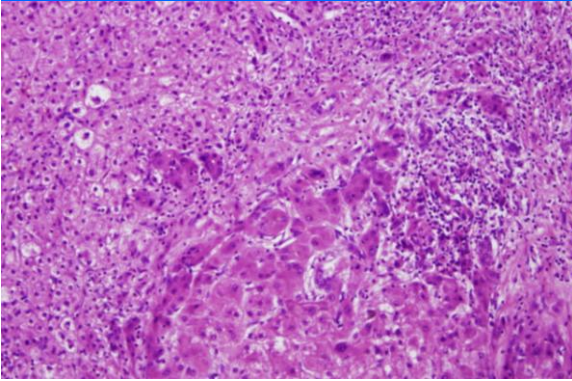
High grade DN vs. early HCC

	HGDN	Early HCC
Small cell change	+	+
Pseudoglands	+	+
Trabeculae	1-3	1-3
Portal tracts	+	+
Unpaired arteries	Few	Few
Reticulin	N or focal	N or focal
Stromal invasion	-	+

- Stromal invasion in HCC**
- Earliest morphological feature of HCC
 - Invasion of neoplastic cells into portal tracts, septa, adjacent parenchyma or blood vessels



Stromal invasion



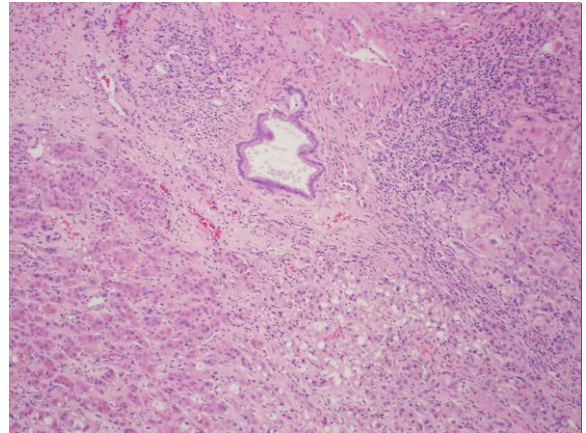
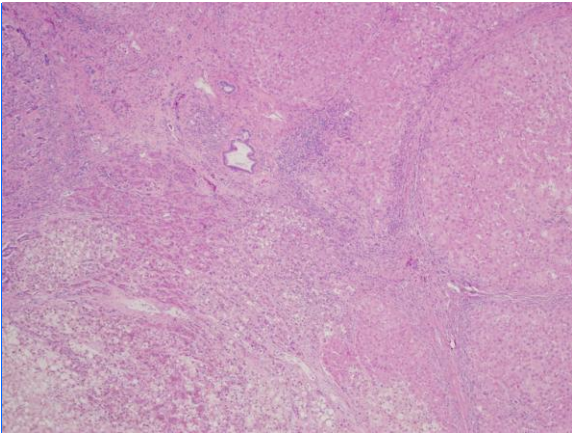
Ductular reaction and stromal invasion

CK7+ DR at nodular interface

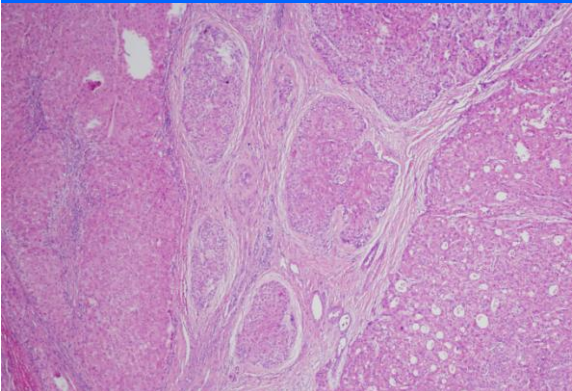
Regenerative	Present
HGDN	Largely present
HCC	Absent or focal

Stromal invasion: no or minimal DR

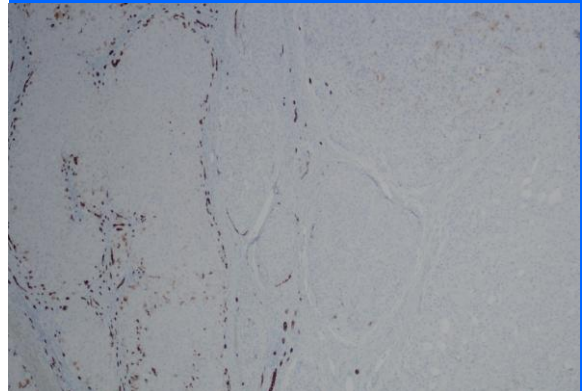
Park, Cancer, 2007



HCC with stromal invasion



CK7+ DR absent in HCC



HGDN vs early HCC

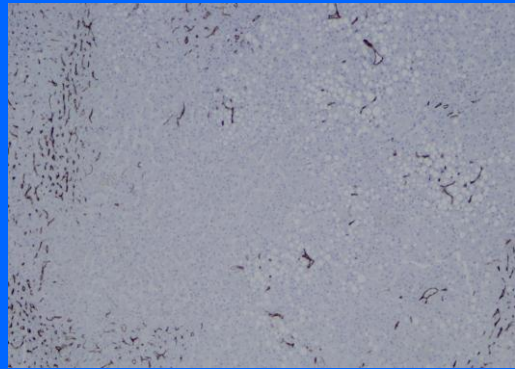
Stromal invasion

- CK7+ ductular reaction

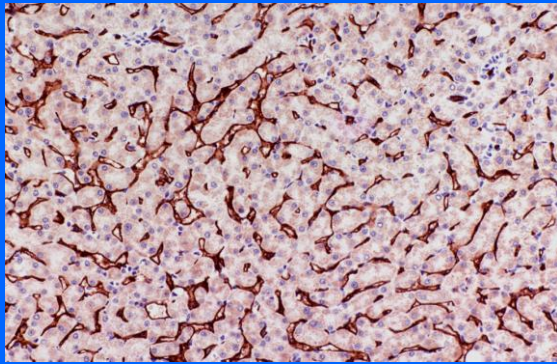
Immunohistochemistry

- CD34
- Heat shock protein 70 (HSP70)
- Glutamine synthetase (GS)
- Glypican-3

CD34 in HGDN: peripheral



CD 34 in HCC: multifocal/diffuse



Immunohistochemistry

- CK7+ ductular reaction
- CD34
- Heat shock protein 70 (HSP70)
- Glutamine synthetase (GS)
- Glypican-3 (GPC-3)

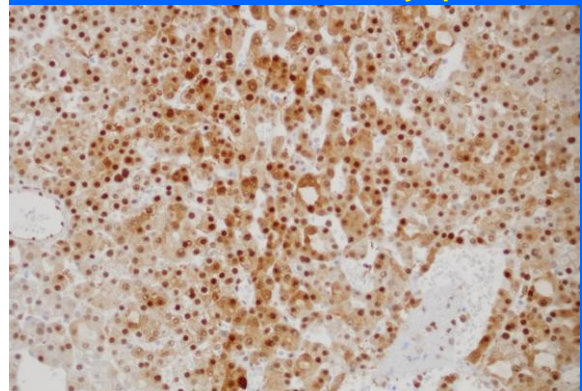
HSP70

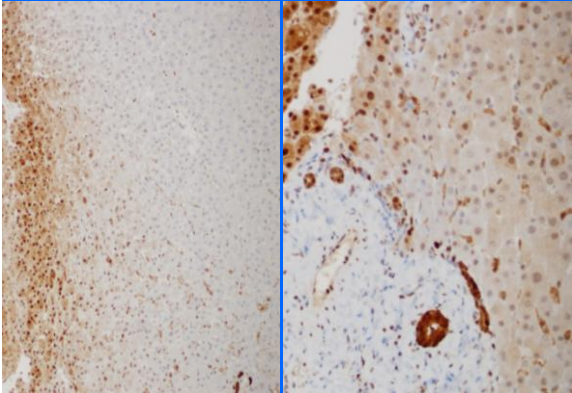
Early HCC and non-cancer liver

- 12,600 genes
- HSP70: most abundantly upregulated in HCC
- Cell cycle progression and apoptosis

Chuma, Hepatology, 2003

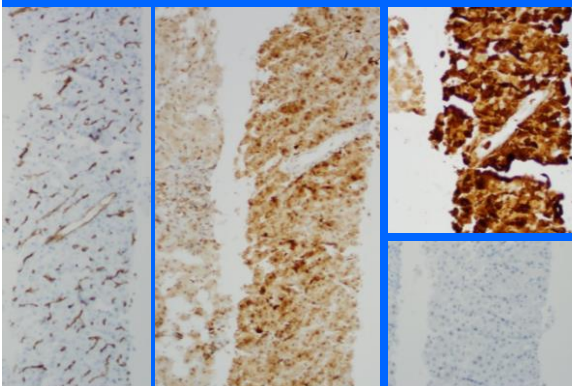
HSP70 in HCC: nuclear and cytoplasmic



HSP70 in adjacent liver: bile duct staining**Combined immunostaining****HSP70, GS and GPC-3**

Tamasso, Hepatol 07	All negative	Any one +	Any two +	All positive
HGDN	72%	28%	0	0
HCC	9%	91%	72%	44%

Tamasso, Hepatol 09	All negative	Any one +	Any two +	All positive
HGDN	78%	22%	0	0
HCC	8%	90%	50%	20%

CD34+**HSP70+****GS+ GPC-****Actual practice**

Marker	Interpretation
HSP70	Often positive in adjacent liver Diagnosis obvious in most cases when positive
GPC-3	Very low sensitivity Rarely helpful
GS	Helpful if diffuse staining

Summary

	High grade dysplastic nodule	Early HCC
Stromal invasion	Absent	Present
CK7+ ductular reaction	Present	Absent in area of invasion
CD34	Patchy	Multifocal or diffuse
HSP70, GPC, GS	<2	≥2

Morphology
Reticulin