2013 Colorado Society of Pathology

Pitfalls in the diagnosis of well-differentiated hepatocellular lesions

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Outline

- Hepatocellular adenoma: new WHO classification
- HCA vs. focal nodular hyperplasia
  HCA vs. well-differentiated HCC

Benign hepatocellular lesions: pre-1990

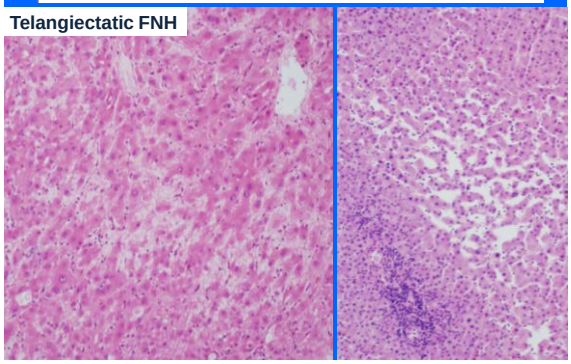
- Focal nodular hyperplasia
- Hepatocellular adenoma

FNH vs. HCA

	Focal nodular hyperplasia	Hepatocellular adenoma
Central scar	Present	Absent
Fibrous septa	Typically present	Typically absent
Nodular architecture	Present	Absent
Ductular reaction	Generally prominent	Absent
Clonality	Polyclonal	Monoclonal

Mod Pathol. 1989 Sep;2(5):456-62.
Multiple focal nodular hyperplasia of the liver associated with vascular malformations of various organs and neoplasia of the brain: a new syndrome.
Wanless IR, Albrecht S, Bilbao J, Frei JV, Heathcote EJ, Roberts EA, Chiasson D.

Telangiectatic FNH



Benign hepatocellular lesions: 1990s

- Focal nodular hyperplasia
  Telangiectatic FNH
- Hepatocellular adenoma

Hepatocellular adenoma

The French Revolution

Telangiectatic FNH

- Clonality studies: Monoclonal
- Protein profiling: cluster with adenomas
- Imaging: resembles adenoma

Reclassified as telangiectatic adenoma

- Ductular reaction
- Fibrous septa with dystrophic arterioles
- Telangiectasia, inflammatory infiltrate

Paradis, Gastroenterology, 2004

Benign hepatocellular lesions: pre-2008

- Focal nodular hyperplasia
- Hepatocellular adenoma

Telangiectatic or variant adenoma

Genotype–Phenotype Correlation in Hepatocellular Adenoma: New Classification and Relationship With HCC

Jessica Zucman-Rossi,[1] Emmanuelle Jeannot,[1] Jeanne Tran Van Nhieu,[2] Jean-Yves Scoazec,[3] Catherine Guettier,[4] Sandra Rebouissou,[1] Yannick Bacq,[5] Emmanuelle Leteurtre,[6] Valérie Paradis,[7] Sophie Michalak,[8] Dominique Wendum,[9] Laurence Chiche,[10] Monique Fabre,[11] Lucille Mellottee,[1] Christophe Laurent,[12] Christian Partensky,[3] Denis Castaing,[4] Elie Serge Zafrani,[2] Pierre Laurent-Puig,[13] Charles Balabaud,[12,14] and Paulette Bioulac-Sage[14,15]

Hepatocellular adenomas are benign tumors that can be difficult to diagnose. To refine their classification, we performed a comprehensive analysis of their genetic, pathological, and clinical features. A multicentric series of 96 liver tumors with a firm or possible diagnosis of hepatocellular adenoma was reviewed by liver pathologists. In all cases, the genes coding for hepatocyte nuclear factor 1α (HNF1α) and β-catenin were sequenced. No tumors were mutated in both HNF1α and β-catenin enabling tumors to be classified into 3 groups, according to genotype. Tumors with HNF1α mutations formed the most important group of adenomas (44 cases). They were phenotypically characterized by marked steatosis ($P < 10^{-4}$), lack of cytological abnormalities ($P < 10^{-6}$), and no inflammatory infiltrates ($P < 10^{-4}$). In contrast, the group of tumors defined by β-catenin activation included 13 lesions with frequent cytological abnormalities and pseudo-glandular formation ($P < 10^{-5}$). The third group of tumors without mutation was divided into two subgroups based on the

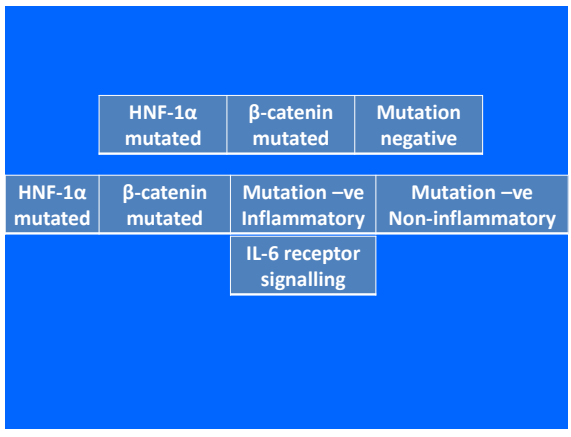
Hepatology. 2006;43:515-24

HCA: WHO classification 2010

HNF-1α inactivated	β-catenin activated	Inflammatory
HNF-1α mutation	β-catenin mutation	JAK/STAT pathway
Women, OC use Familial	40% in men Androgens	Women (OCs), men Obesity, diabetes
Marked steatosis, no atypia	Pseudoacinar, cytologic atypia, small cell change	Inflammation, sinusoidal dilatation, ductular reaction
HCC rare		HCC rare

Mutation-negative cases

- Inflammatory: Similar to telangiectatic adenoma
- Non-inflammatory: no inflammation or sinusoidal dilatation



Hepatocellular adenoma

The French Revolution
2006: part 2

Hepatocellular Adenoma Subtype Classification Using Molecular Markers and Immunohistochemistry

Paulette Bioulac-Sage,[*1,2] Sandra Rebouissou,[*3,4] Cristel Thomas,[3,4] Jean-Frédéric Blanc,[2,5] Jean Saric,[6] Antonio Sa Cunha,[6] Anne Rullier,[1,2] Gaëlle Cubel,[2] Gabrielle Couchy,[3,4] Sandrine Imbeaud,[7] Charles Balabaud,[2,5] and Jessica Zucman-Rossi[3,4]

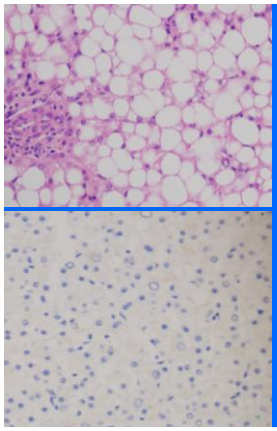
Hepatocellular adenomas (HCA) with activated β-catenin present a high risk of malignant transformation. To permit robust routine diagnosis to allow for HCA subtype classification, we searched new useful markers. We analyzed the expression of candidate genes by quantitative reverse transcription polymerase chain reaction QRT-PCR followed by immunohistochemistry to validate their specificity and sensitivity according to hepatocyte nuclear factor 1 alpha (HNF1α) and β-catenin mutations as well as inflammatory phenotype. Quantitative RT-PCR showed that *FABP1* (liver fatty acid binding protein) and *UGT2B7* were downregulated in HNF1α-inactivated HCA ($P \leq 0.0002$); *GLUL* (glutamine synthetase) and *GPR49* overexpression were associated with β-catenin–activating mutations ($P \leq 0.0005$), and *SAA2* (serum amyloid A2) and *CRP* (C-reactive protein) were upregulated in inflammatory HCA ($P = 0.0001$). Immunohistochemistry validation confirmed that the absence of liver-fatty acid binding protein (L-FABP) expression rightly indicated HNF1α mutation (100% sensitivity and specificity), the combination of glutamine synthetase overexpression and nuclear β-catenin staining were excellent predictors of β-catenin–activating mutation (85% sensitivity, 100% specificity), and SAA hepatocytic staining was ideal to classify inflammatory HCA (91% sensitivity and specificity). Finally, a series of 93 HCA was unambiguously classified using our 4 validated immunohistochemical markers. Importantly, new associations were revealed for inflammatory HCA defined by SAA staining with frequent hemorrhages ($P = 0.003$), telangiectatic phenotype ($P < 0.001$), high body mass index, and alcohol intake ($P \leq 0.04$). Previously described associations were confirmed and in particular the significant association between β-catenin–activated HCA and hepa-

Hepatology. 2007 ;46:740-8

HCA: immunohistochemistry

HNF-1α mutated	Inflammatory	β-catenin mutated
Fatty acid binding protein (FABP)	C-reactive protein (CRP) Serum amyloid associated protein (SAA)	β-catenin Glutamine synthetase (GS)
FABP negative	CRP+ SAA+	Nuclear β-catenin Diffuse GS

Unclassified (5-10%): no known defining features



HNF1α mutated

- Women
- Steatosis
- Atypia, risk of HCC: minimal
- Fatty acid binding protein absent

HCA: immunohistochemistry

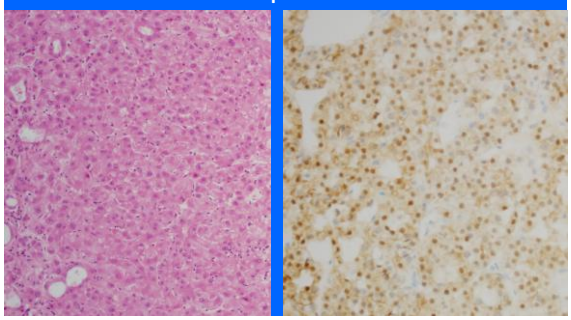
HNF-1α mutated	β-catenin mutated	Inflammatory	Unclassified
-Fatty acid binding protein (FABP)	β-catenin Glutamine synthetase (GS)	-C reactive protein (CRP) -Serum amyloid associated protein (SAA)	No defining features
FABP negative	Nuclear β-catenin Diffuse GS	CRP+ SAA+	

β-catenin mutated

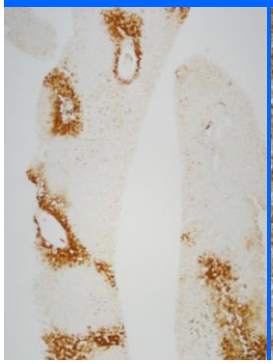
40% men

Cytologic atypia, frequent association with HCC

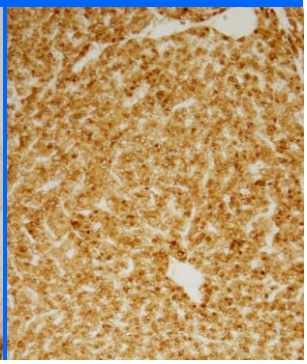
Nuclear translocation of β-catenin



Normal liver: perivenular GS



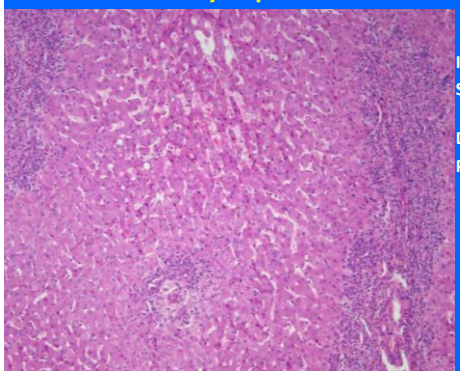
β-catenin-activated adenoma: diffuse GS



HCA: genotype-phenotype

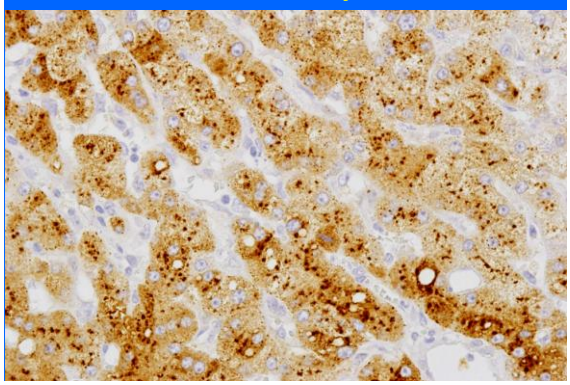
HNF1-mutated	Beta-catenin mutated	Inflammatory
-Fatty acid binding protein (FABP)	-Beta-catenin -Glutamine synthetase (GS)	-C reactive protein (CRP) -Serum amyloid associated protein (SAA)
FABP negative	Nuclear beta-catenin Diffuse GS	CRP+ SAA+

Inflammatory hepatocellular adenoma



- Inflammation
- Sinusoidal dilatation
- Ductules
- Pseudo-PT

SAA in inflammatory adenoma



HCA: WHO classification 2010

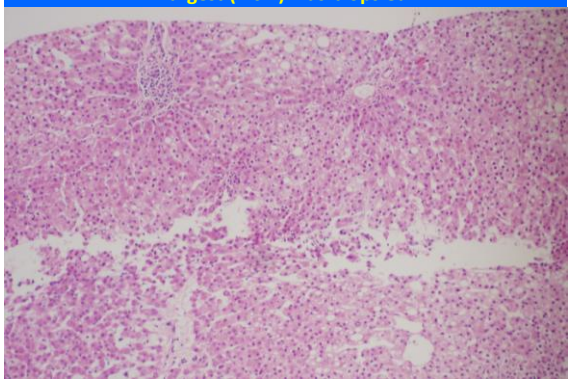
HNF-1α inactivated	Inflammatory	β-catenin activated
35-50%	40-50%	10%
Women, OC use	Women (OCs), men Obesity, diabetes	40% in men Androgens, glycogen storage disease
Marked steatosis, no atypia	Inflammation, sinusoidal dilatation, ductular reaction	Pseudoacinar, small cell change
HCC rare	HCC rare	HCC 40%
FABP negative	SAA positive CRP positive	Nuclear β-catenin Diffuse GS

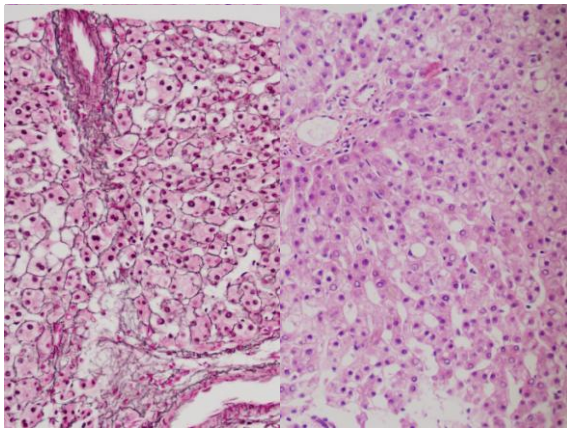
Unclassified (5-10%): no known defining features

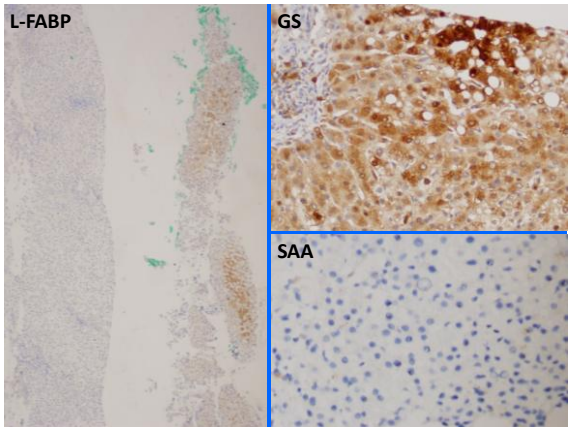
HCA classification: immunohistochemistry

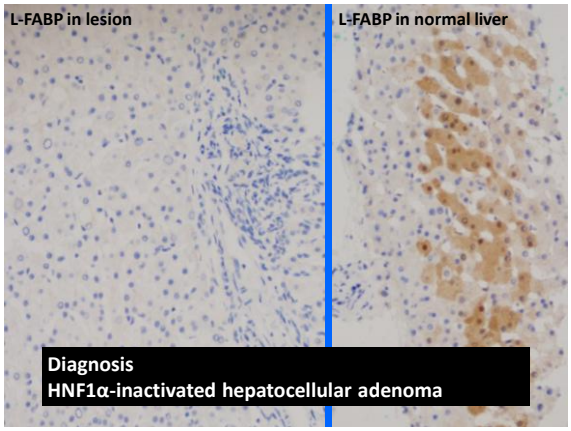
	L-FABP	SAA CRP	β-catenin	GS
HNF-1α inactivated	negative	negative	membranous	peri-vascular
Inflammatory	cytoplasmic	positive	membranous	peri-vascular
β-catenin activated	cytoplasmic	negative	nuclear	diffuse
Unclassified	cytoplasmic	negative	membranous	peri-vascular

Case 1: Obese 42/F with multiple (>15) liver lesions; largest (4 cm) was biopsied









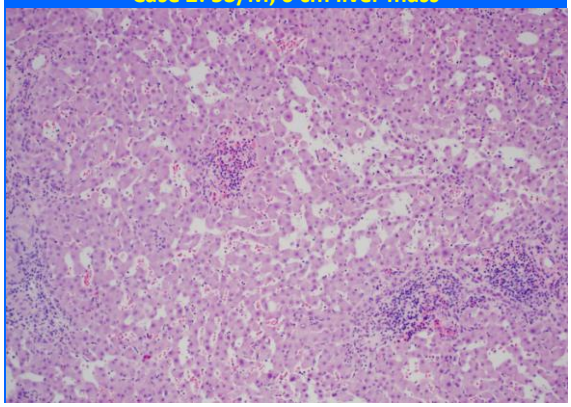
Hepatic adenomatosis

- By definition, ≥10 adenomas
- Young women
- Most are HNF1α-inactivated or inflammatory
- Pathogenesis
  - Obesity, less strong association with OCs
  - Germ line HNF1α mutations

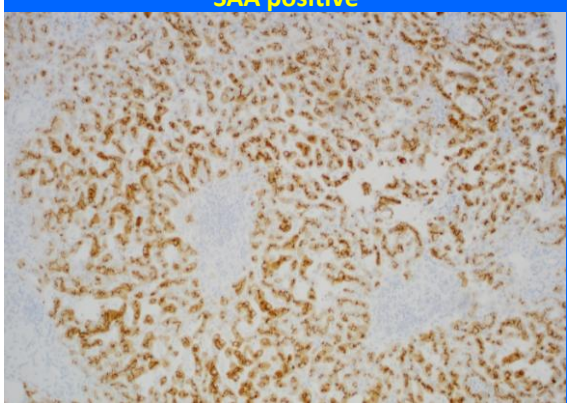
Management of HCA

Management	Tumor characteristics
Resection	Women: solitary HCA ≥5 cm Men (?women>50 years): all cases β-catenin activated Glycogen storage disease
Conservative with annual surveillance	Solitary HCA <5 cm
Conservative with annual surveillance	Multiple HCAs or adenomatosis, depending on size

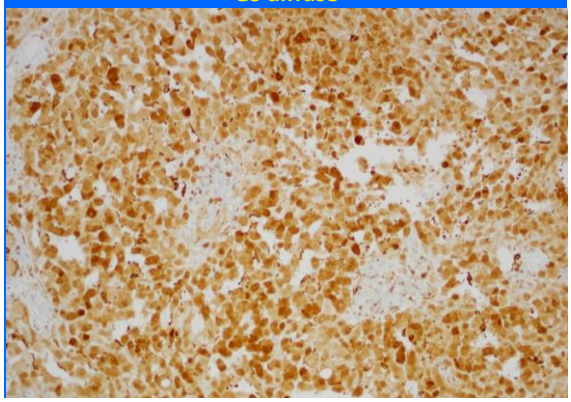
Case 2: 53/M, 6 cm liver mass



SAA positive



GS diffuse



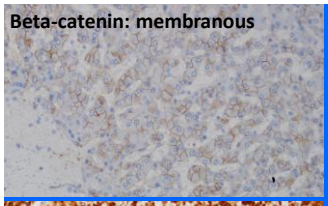
Questions

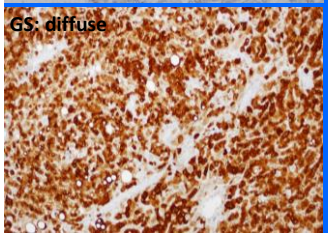
β-catenin membranous, GS diffuse

- Does this represent β-catenin activation?

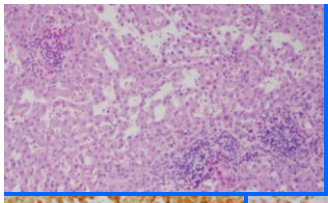
SAA+ and GS diffuse

- Is this inflammatory HCA or β-catenin activated HCA?

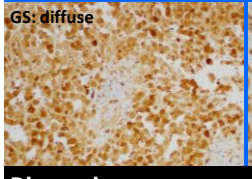


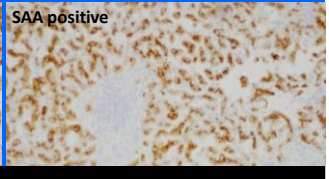


- 15% of β-catenin activated HCA
- Most have β-catenin mutation
- Nuclear β-catenin focal or below threshold



Inflammatory HCA
10% have β-catenin activation





Diagnosis
Inflammatory HCA with β-catenin activation

Immunostaining variations in HCA

Variation	Interpretation
Membranous β-catenin, diffuse GS	β-catenin activation
SAA positive, diffuse GS, ± nuclear β-catenin	β-catenin activation
Nuclear β-catenin in few tumor cells	β-catenin activation
LFABP patchy; negative in some areas	Does not support HNF1α-inactivated HCA
Morphology not typical of inflammatory HCA, SAA positive	Inflammatory HCA
Inflammatory HCA by morphology, SAA negative	Inflammatory HCA

Outline

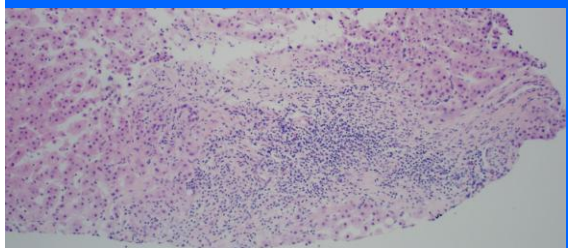
- Hepatocellular adenoma: new WHO classification
- HCA vs. focal nodular hyperplasia
  HCA vs. well-differentiated HCC

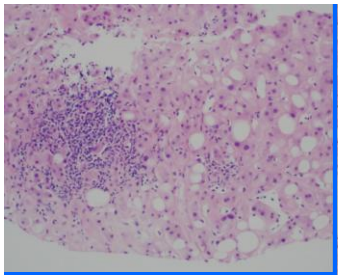
HCA vs. FNH: clinical significance

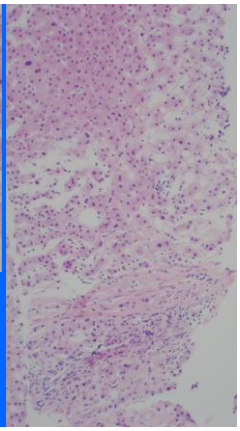
Focal nodular hyperplasia	Hepatocellular adenoma
Non-neoplastic	Neoplastic
No surgery in most cases	Surgery if high risk features: Male, size >5 cm
Large, symptomatic, atypical features	Risk of hemorrhage, associated HCC

Case 3

- 32 year old woman on OCs
- Ultrasound for workup of abdominal pain
- 5 cm liver mass, suggestive of FNH







Inflammation
Arterioles
Few ductules
Sinusoidal dilatation

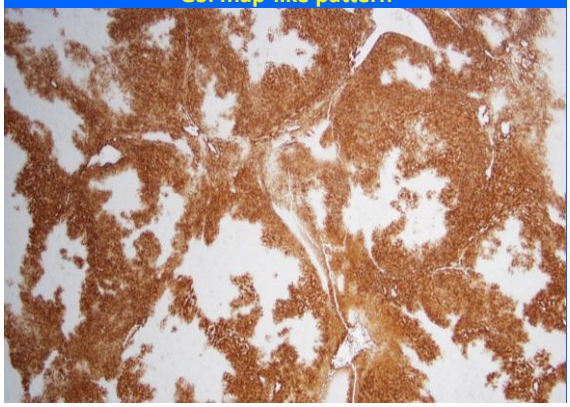
FNH or inflammatory HCA

For FNH	Imaging favors FNH Aberrant arterioles in fibrous septa Ductular reaction
For inflammatory HCA	Sinusoidal dilatation Inflammation

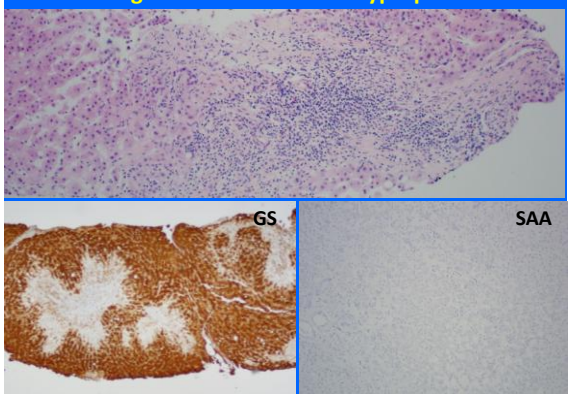
FNH or inflammatory HCA

Immunostain	Inflammatory HCA	FNH
Serum amyloid A (SAA)	Moderate to strong	Absent/focal
Glutamine synthetase (GS)	β-catenin activated: diffuse Others: Perivascular/patchy	Map-like

GS: map-like pattern



Diagnosis: Focal nodular hyperplasia



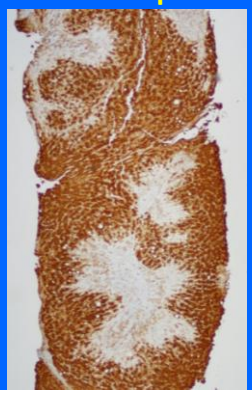
Histologic feature	FNH	Inflammatory HCA	p value
Sinusoidal dilatation	18%	83%	<0.001
Inflammation	40%	60%	0.08
Steatosis	21%	57%	0.001
Fibrous bands	90%	26%	<0.001
Ductular reaction	83%	43%	<0.001

Joseph/Kakar, USCAP meeting 2011

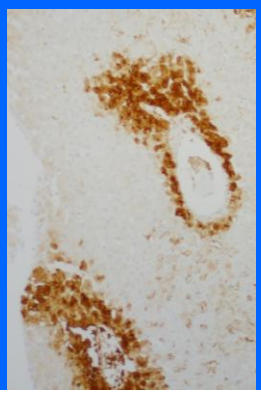
FNH vs. IHCA: challenges in interpretation

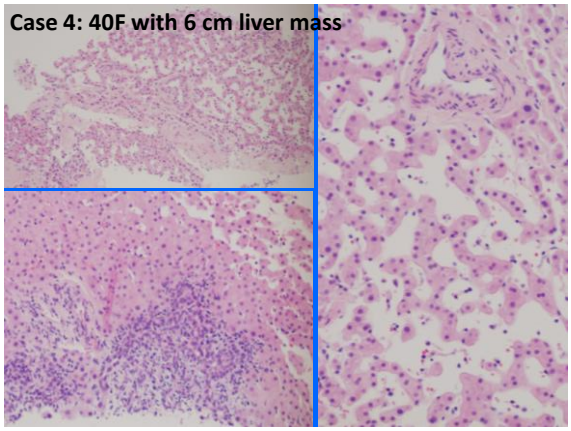
- Map-like GS patterns on needle biopsies
- Map-like vs. diffuse GS pattern
- 'Pseudo map-like' pattern
- SAA in FNH and adjacent liver
- Use of CRP in addition to SAA

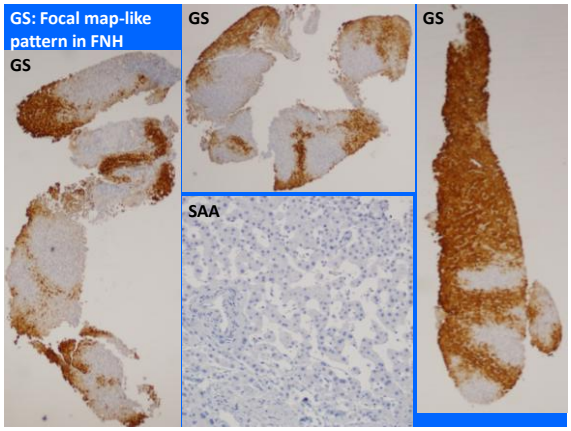
GS: map-like

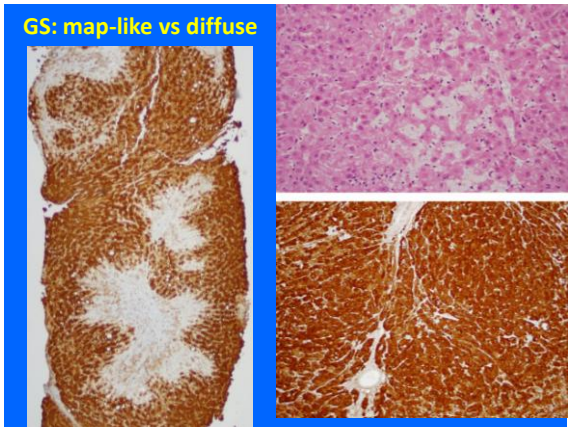


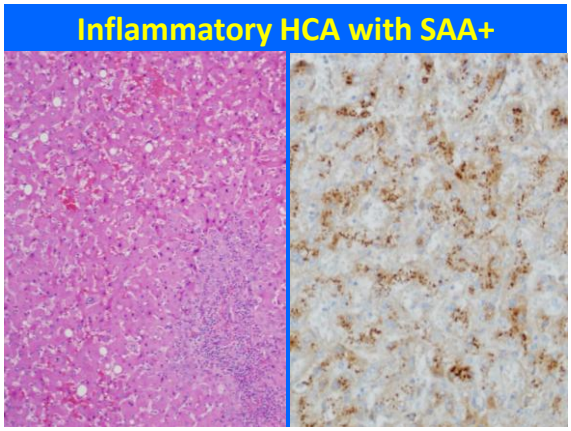
GS: normal

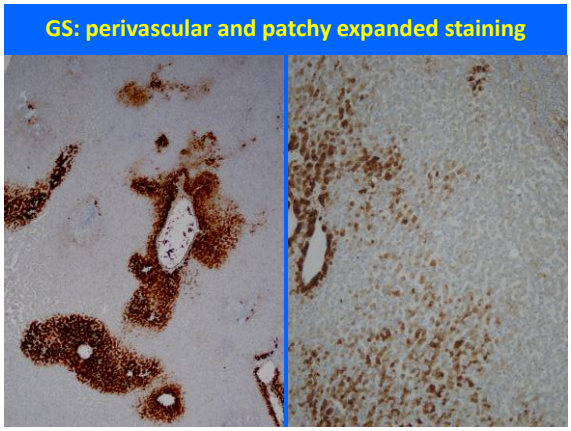


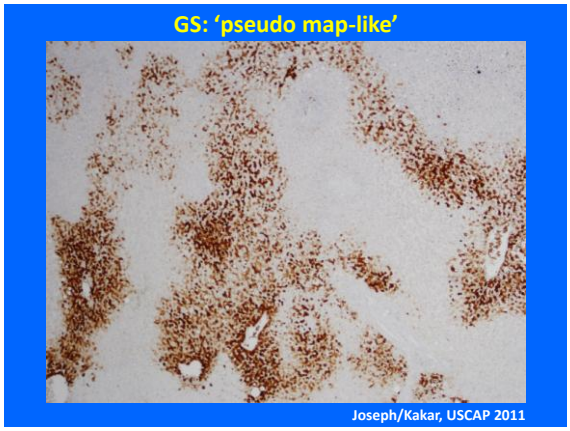

Case 4: 40F with 6 cm liver mass


GS: Focal map-like pattern in FNH
GS
GS
SAA
GS


GS: map-like vs diffuse

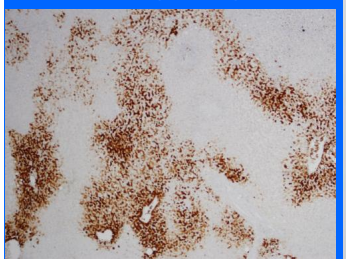

Inflammatory HCA with SAA+

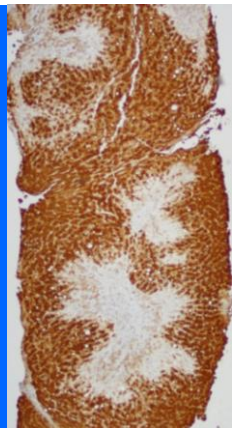

GS: perivascular and patchy expanded staining


GS: 'pseudo map-like'
Joseph/Kakar, USCAP 2011

GS: "Pseudo map-like"

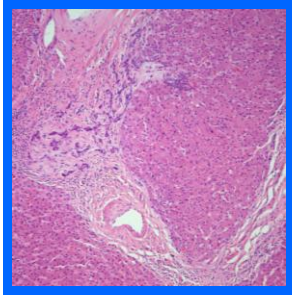
- Confined to periphery
- Patchy and variable
- Broad hepatocyte groups with uniform strong staining absent



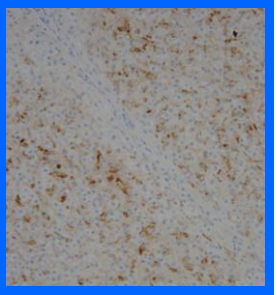


Case 5: 37F with 4 cm liver mass

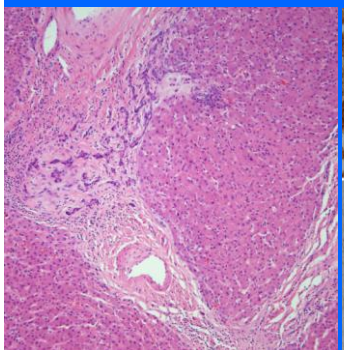
HE stain



SAA+

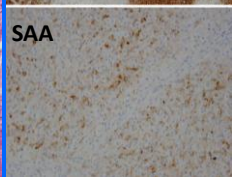


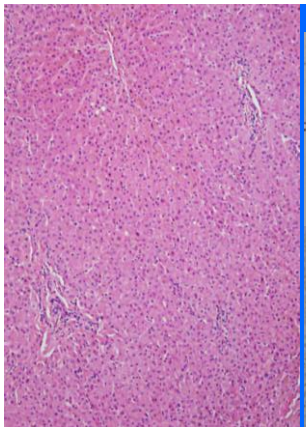
FNH with map-like GS and SAA+



GS

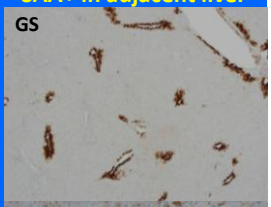
SAA



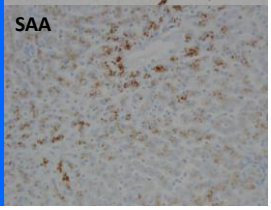


SAA+ in adjacent liver

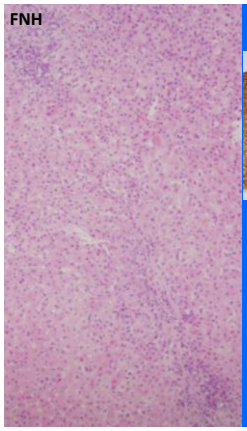
GS



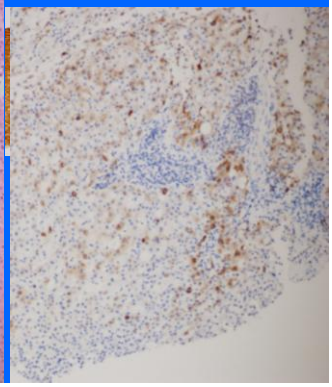
SAA



FNH



Role of CRP



SAA and CRP in IHA and FNH

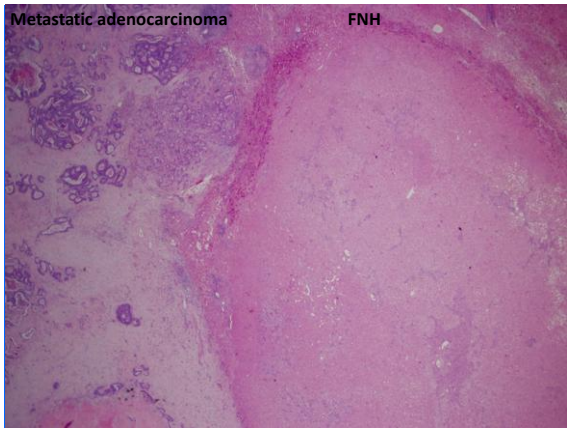
	IHA	FNH
SAA+ (>50%)	80%	5-10%
CRP+ (>50%)	100%	20-25%

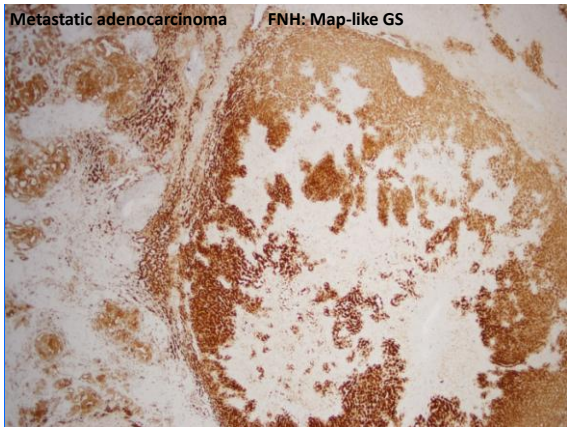
Joseph/Kakar/Ferrell, Mod Pathol 2013

FNH-like lesion

Morphology and GS staining similar to classic FNH

- Adjacent to tumors
- Cirrhosis
- Vascular abnormalities

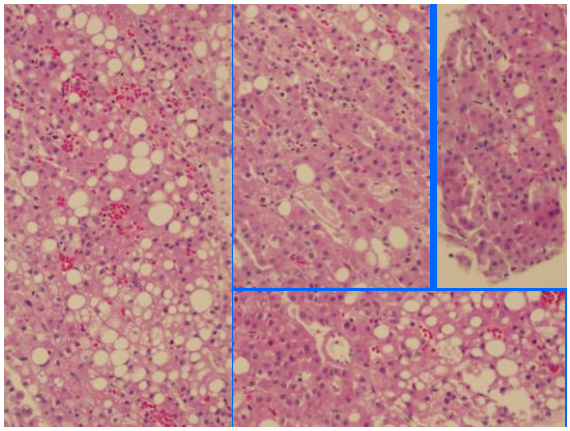




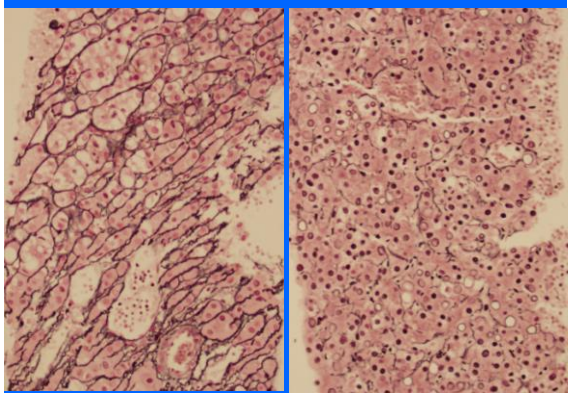
HCC vs. hepatocellular adenoma

Case 6 *

- 60/M with diabetes and renal failure
- CT showed a 3.9 cm mass, concerning for HCC or metastatic carcinoma, but appearance was nonspecific and can represent hepatocellular adenoma



Reticulin stain



International Working Party

	Adenoma	WD-HCC
Wide plates (≥ 3)	-	+
Small cell change	-	+
Cytologic atypia	-	-/+
Reticulin	Normal	Fragmented

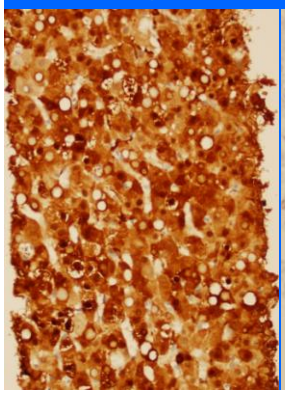
Ferrell et al, Hepatology 1995

Combined immunostaining

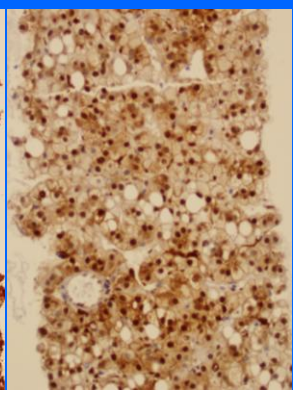
HSP70, GS and GPC-3: HCA vs. HCC

Study	Results
Lagana et al, Appl Immunohistochem Mol Morph, 2012	HSP70, GPC-3 <50% sensitivity 100% specific GS 80% sensitivity 50% specific
Nguyen/Kakar, USCAP, 2012	HSP70 in 13% of HCA Overall limited utility

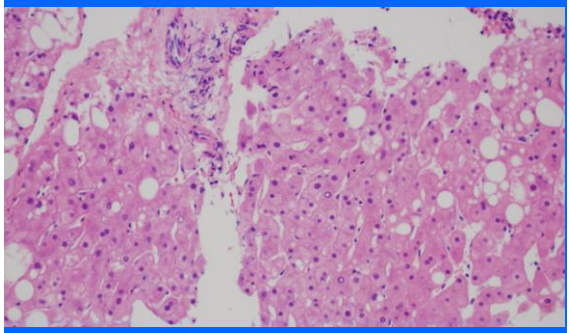
Glutamine synthetase



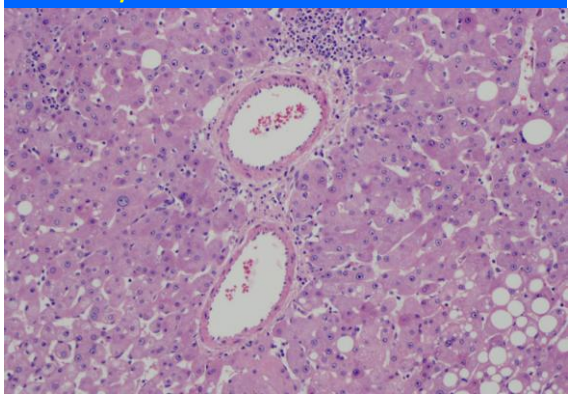
HSP 70



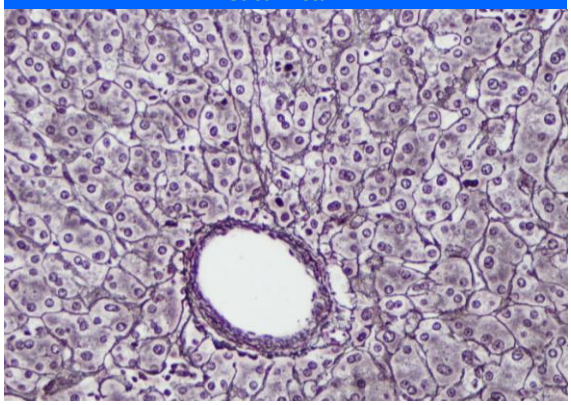
Case 6: 58/M with 5 cm hepatic mass: biopsy



58/M with 5 cm liver mass: resection



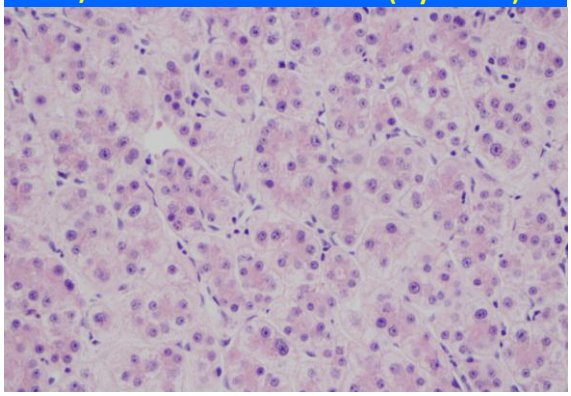
Reticulin stain



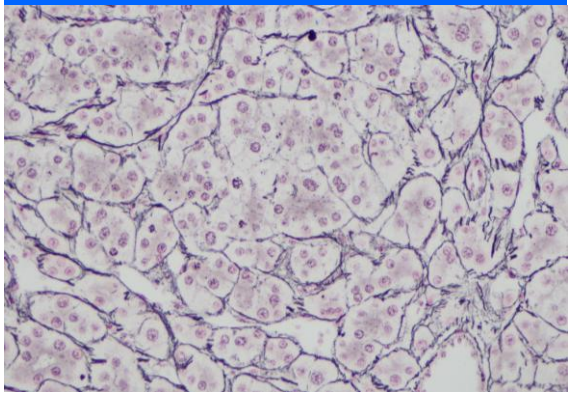
HCA or HCC

For HCA	Thin cell plates Lack of cytologic atypia Intact reticulin
For HCC	Age >50 years Male gender

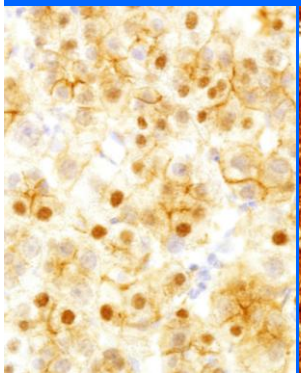
61/M with 3.0 cm liver mass (3 yrs later)



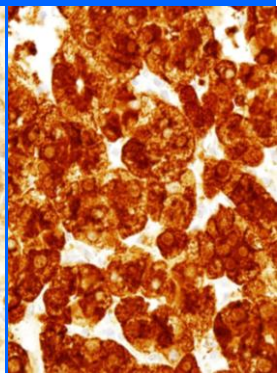
Well-differentiated HCC



Beta-catenin



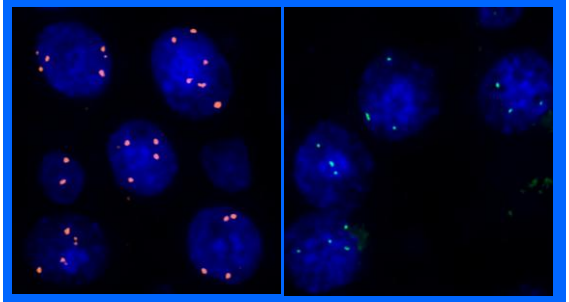
GS



FISH

CEP1 gain

CEP8 gain



Kakar/Ferrell, Histopathol, 2009, FISH by JP Grenert, UCSF

β-catenin activated HCA, or β-catenin activated HCC

Morphology*	HCC*	Cytogenetics**
Atypia: 70%	Concurrent /follow-up: 40%	Chromosomal changes: 60%

*B Sage, Hepatol 2008
**Evason/Kakar, Human Pathol 2012

What makes a tumor malignant?

Source	Definition
Webster Medical Dictionary	Ability to invade local tissues Ability to spread to distant sites (metastasize)
Stedman Medical Dictionary	
Dorland Medical dictionary	
Robbins' Pathology	

Is β-catenin activated 'HCA' malignant?

- Local invasion (recurrence) Yes
- Metastasis Yes
- Pathologic features Yes/no
- Supportive evidence Yes

High risk factors

Focal atypical morphology	Age/gender	Immunostaining
Pseudoacinar	Male gender	Nuclear β-catenin
Small cell change	Older age (>50 yrs)	Diffuse GS
Thick plates		
Reticulin loss		

Well-differentiated hepatocellular neoplasm with atypical features, HCC or β-catenin activated hepatocellular adenoma

Hepatocellular adenomas in a large community population 2000-2010: reclassification per current WHO classification and results of long-term follow-up

GL Genrich[1], N Shafizadeh[2], L Ferrell[1], S Kakar[1]
[1]Pathology, UCSF Medical Center, San Francisco, CA.
[2]Pathology, Southern California Permanente Medical Group, Woodland Hills, CA

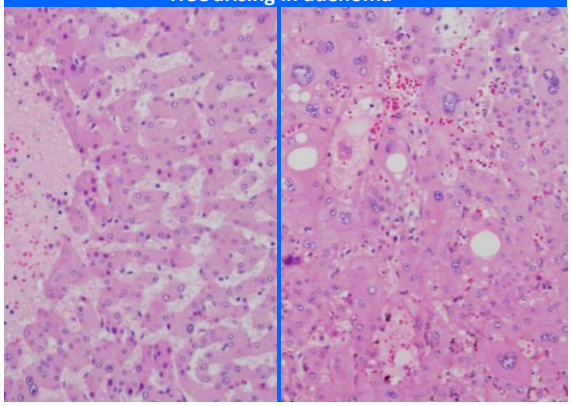
Table 3: Comparison of adenoma subtype in this study with 2 French studies

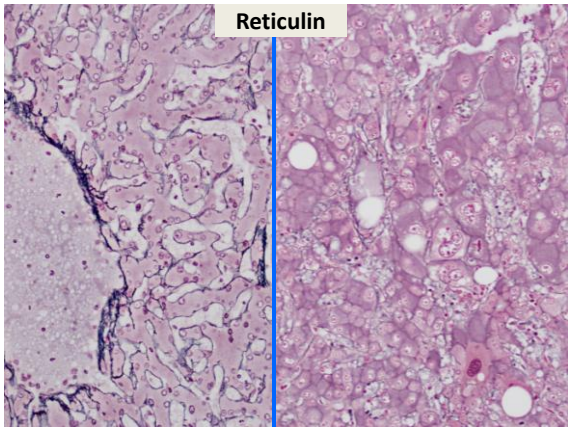
	A. Present study (n=28)	B. Bioulac-Sage, Hepatology, 2007 (n=93)	C. Bioulac-Sage, Am J Surg Path, 2012 (n=137)	p value A vs. B/A vs. C
HNF1-α inactivated	29%	33%	22%	0.16/0.12
Inflammatory	32%	40%	53%	0.09/0.007
β-catenin activated, non-inflammatory	0	17%	2%	0.01/0.57
IHA with β-catenin activation	3%	2%	11%	0.55/0.16
Unclassified	36%	8%	13%	0.001/0.004

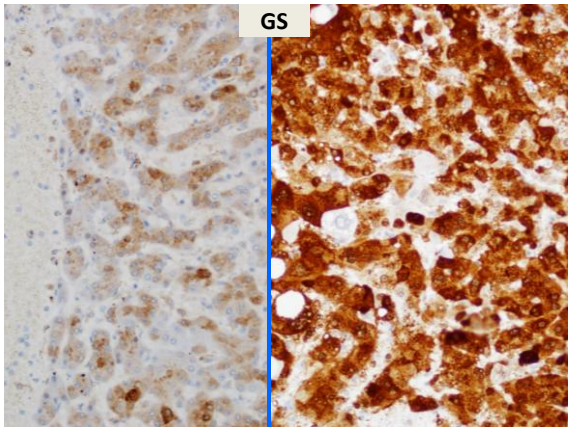
CONCLUSIONS

Most β-catenin activated hepatocellular tumors can be diagnosed as HCC with careful attention to morphology and reticulin staining pattern, especially in resection specimens. *

HCC arising in adenoma







HCC in adenoma

Adenoma	Presence of HCC
Stoot, HPB, 2010 studies from 1970-2009	68/1635= 4%
Size >5 cm <5 cm	>95% ~4%

Minimum stains

Stain	Interpretation
Reticulin	Loss: HCC
GS	Diffuse: β-catenin activation Map-like: FNH Other patterns: HCA
SAA	Inflammatory HCA

Well-differentiated hepatocellular lesion

Stain	Utility
Reticulin	HCC
SAA	Inflammatory HCA
GS	Map-like: FNH Diffuse: Beta-catenin mutated

Stain	Utility
LFABP	HNF1-alpha inactivated HCA
Beta-catenin	Beta-catenin mutated
CRP	Inflammatory
Glypican-3, HSP70	HCC

Imaging features

	FNH	Inflammatory HCA
Central scar	Present	Absent
Contrast CT enhancement	Early homogenous	Heterogeneous and persistent
MRI TI-weighted	Hypointense	Hyperintensity
MRI T2-weighted	Hypointense	Strong hyperintensity

Feature	Pitfall	Approach
SAA staining in peritumoral liver in needle biopsy that missed the lesion	Misinterpreted as evidence of IHA, especially when other peritumoral features like inflammation, sinusoidal dilatation and ductular reaction are also present	SAA+ is not specific for IHA. Interlobular bile ducts and absence of diffuse CD34 staining can help in confirming that the biopsy comprises non-neoplastic liver.
SAA negative in a lesion that shows typical features of IHA	Misinterpreted as absolute evidence against IHA	SAA can be negative in 5-10% of IHA. Imaging and absence of map-like GS staining is needed to confirm IHA in these cases.
SAA positive in a lesion that shows typical features of FNH	Misinterpreted as IHA	Focal SAA can be seen in 15% of FNH. Map-like GS confirms FNH irrespective of SAA .
Perivenous and patchy GS in a lesion that shows typical features of IHA	Misinterpreted as map-like pattern of FNH	This staining is common in IHA. It is distinguished from map-like pattern by (i) lack of anastamosing pattern of staining. (ii) staining is heterogeneous and weak compared to homogeneous strong staining in map-like pattern.
Diffuse GS staining	Misinterpreted as map-like pattern of FNH	Diffuse GS is seen in beta-catenin-activated tumors and does not have areas of periseptal sparing of map-like pattern. Most also show nuclear beta-catenin.
Pseudo-map like GS pattern	Misinterpreted as map-like pattern of FNH	This is seen at the periphery of IHA and 10% of FNH . When seen in biopsies, it is more likely to be FNH. Diagnosis on needle biopsy may remain indeterminate if imaging and SAA are not helpful.