Role of pathology in neoplastic liver disease

P. Schirmacher

Institute of Pathology, University of Heidelberg
Role of Diagnostic Pathology

- Diagnosis of focal lesion (biopsy)
  - Diagnosis, [grading]
  - Additional analyses (targets, markers)
- Resection specimen (partial hepatectomy, Ltx)
  - Confirmation of diagnosis, Grading
  - Extension of disease (Staging)
  - Resection margins
Biopsy diagnosis of focal liver lesions

- **Primary hepatic neoplasias**
  - **Epithelial**
    - **Hepatocellular**
      - Benign (liver cell adenoma, FNH, Dysplastic Nodules)
      - Malignant (HCC, hepatoblastoma)
    - Cholangiolar
      - Benign (Bile duct adenoma, biliary cystadenoma)
      - Malignant (CCC, biliary cystadenocarcinoma)
  - **Mesenchymal**
    - Benign (haemangioma etc.)
    - Malignant (angiosarcoma, other rare sarcomas)

- **Metastases** (confirmation, target analyses)
  - Epithelial (breast, lung, gastrointestinal)
  - Mesenchymal (GIST)

- **Non-neoplastic lesions** (IPT, abscess, hamartomas, etc.)
Macroscopic typing of HCC
(Eggel, 1910; Okuda et al., 1984)

No relevance in regard to pathogenesis, prognosis and therapy
Staging of HCC (2002)

TNM-Classification

T1  solitary tumor without vascular invasion
T2  solitary tumor with vascular invasion or multiple tumors ≤ 5 cm diameter
T3  multiple tumors > 5 cm diameter or tumor invasion of major veins
T4  tumor(s) with invasion of adjacent organs or perforation of visceral peritoneum

Stage Grouping

Stage I  T1  N0  M0
Stage II  T2  N0  M0
Stage IIIA  T3  N0  M0
Stage IIIB  T4  N0  M0
Stage IIIC  any T  N1  M0
Stage IV  any T  any N  M1

Therapeutic (surgery) and limited prognostic relevance
Microscopic HCC-typing
(AFIP [n = 1348])

- Trabecular (micro-/macro-) 61 %
- Compact (solid) 17.1 %
- Mixed (incl. HCC/CCC) 11.5 %
- Fibrolamellar 5.5 %
- Pseudoglandular 4.5 %
- Scirrhous 0.2 %

No relevance in regard to prognosis, therapy, and pathogenesis (questionable exception: fibrolamellar type)
Grading of HCC

• Edmondson and Steiner (1954) (UICC 2002)
• Nzeako et al. (1995) (= AFIP)
• General UICC/WHO grading

Problems:
• Questionable relevance in regard to prognosis; irrelevant for therapy and pathogenesis
• No grading consensus; different from other carcinomas; poor grading decision of UICC
Grading of HCC (AFIP)

G1                     G2                        G3             G4

G1  G2  G3  G4
Standard Criteria of HCC

- Moderate to high grade atypia
- Trabecular disarray
- Invasion (vascular or interstitial)
Ambiguous nodules
Chronic liver damage

- Viral hepatitis
- Genetic disorders
- Chronic alcohol abuse

Dedifferentiation of original hepatocytes?

Stem cell activation?

Small cell proliferation

Dysplastic foci

- Low grade
- High grade

Borderline lesions

Early HCC

Fully developed HCC

Cirrhosis

Fibrosis

Invasion

Tumor growth

Metastasis

Focus formation

Size increase

T
Hepatocarcinogenesis

Dysplastic Nodule-HCC-Sequence

not

Adenoma-HCC-Sequence
Morphological differential diagnosis of highly differentiated hepatocellular tumors

- Hepatocellular Carcinoma (HCC)(G1)
- Dysplastic Nodule (DN)
- Liver Cell Adenoma (LCA)
- Focal Nodular Hyperplasia (FNH)
- Cirrhotic Nodule, Macroleucent Nodule (MRN)
Aspects of Differential Diagnosis

• Histomorphology of the lesion
• Histology of the non-tumorous liver
• Clinical/serological data
• Imaging data
• Quality parameters (amount/ preservation of biopsy tissue, diagnostic work-up)
Focal Nodular Hyperplasia (FNH)
FNH – a reactive vascular lesion
Histomorphology of FNH

- atypical vessels
- no capsule
- small hepatocytes
- scar lymphocytes
- ductular proliferates

*
Liver Cell Adenoma
Liver Cell Adenoma
# Matrix-diagnosis

## FNH/Adenoma

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<th>FNH</th>
<th>Liver Cell Adenoma</th>
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<tbody>
<tr>
<td>• gender: m ~ f</td>
<td>• gender: f &gt;&gt; m</td>
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<tr>
<td>• age: all ages</td>
<td>• age: 3rd/4th decade</td>
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<td>• non-tumorous liver: normal</td>
<td>• non-tumorous liver: normal</td>
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<td>• 1/3 of cases: multiple lesions</td>
<td>• mostly singular lesion</td>
</tr>
<tr>
<td>• History: Steroids, no HCC-risk factors</td>
<td>• History: Steroids, no HCC-risk factors</td>
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</table>
Matrix-diagnosis – HCC/DN

- gender: m > f
- age: 6th-7th decade
- Patient from endemic area?
- Predisposing diseases: HBV, HCV, gen. haemochromatosis, alcohol
- Non-tumorous liver: chronic hepatitis, cirrhosis
Premalignant hepatocellular lesions


1. Liver cell dysplasia [Dysplastic Focus]
2. Dysplastic Nodule (Adenomatous Hyperplasia)
   - low grade
   - high grade

**Dysplastic Nodule**

1. **Size**: 0.1 - 1.5 (2) cm
2. **Morphology**:
   - clonal aspect
   - mild atypia
   - absence of malignancy criteria
Dysplastic Nodule

Low-grade to high-grade transition
Highly differentiated HCC

'nodule in nodule'

interstitial invasion

trabecular disarray

pseudoglandular structures capsule
Biopsy-criteria of highly differentiated HCC
(Kondo et al., 1989)

- Nuclear crowding (smaller cell size)
- Increased cytoplasmic basophilia
- Microacinar structures

2 criteria: 80% possibility of HCC!
HCC is a good carcinogenesis model

1. Clinical relevance (at least 5th most frequent malignant tumor, rising incidence)
2. Well defined etiology
3. Defined premalignant lesions
4. Good animal and cell culture models

But HCC is the most frequent orphan malignancy worldwide!
Role of Experimental Pathology

- Pathogenesis (mechanisms) of hepatocarcinogenesis
  - Targets for primary and secondary prevention
  - Targets for therapeutic approaches
  - Independent prognostic markers
  - Markers for differential tissue diagnosis

- Functional evaluation
  - Cell culture
  - Animal models

- Assays for tissue diagnosis (biopsy tissue)
  - Clinical trials
  - Patient care
CGH in HCCs and DNs

HCCs

DNs

Niketeghad et al., Br J Cancer 2001
Metaanalysis of genomic imbalances in human HCCs

### General analyses of genomic imbalances (n=719)

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### HBV-specific imbalances (n=386)

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Moinzadeh et al., (2005) Br J Cancer
1q gains in hepatocarcinogenesis

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- frequent in all cancers
- early lesion in HCC
- associated with CIN phenotype

Moinzadeh et al., (2005) Br J Cancer
Chromosomal progression model of HCC
(30 DN and 785 HCCs from 31 CGH-studies)

Moinzadeh et al., (2005) Br J Cancer
Expression profiling in HCCs

Tissue cDNA-Array Clustering Data analyses

Correlations (morphology, clinical data)

Markers, molecular subtyping

Therapeutic Targets

Functional analyses
cDNA-microarray analyses in human HCCs

Breuhahn et al., Cancer Res (2004)
Apoptosis and tumor-infiltrating lymphocytes in HCC

Breuhahn et al., Cancer Res (2004)
Potential targets

IGF-II  Tyrosine kinases
COX-2  p53
Ep-CAM  Proteasome
IGF-II overexpression in SV40Tag-induced HCCs

IGF-II
Histone H3-2
Bright field

Schirmacher et al., Cancer Res 1992
IGF-II secretion of human liver tumor cells

SK-hep-1

Hep G2

Hep 3B

HuH7

Lund et al., Cancer Lett. 2004
Reduced growth of HCC-cells after IGF-II inhibition

Lund et al., Cancer Lett. 2004
IGF-II increases chemotherapy response in HCC-cells

Lund et al., Cancer Lett. 2004
IGF-II-suppression by IFNγ in HCC-cells

Breuhahn et al., Cancer Res (2004)
Ep-CAM expression in HCC

HCC (G2) Ep-CAM positive

HCC (G2) Ep-CAM negative

Chronic hepatitis C (strong)

Chronic hepatitis C (weak)

PBC Ep-CAM negative

Therapeutic option: anti Ep-CAM treatment (specific antibody; 15% positivity in HCCs)

Breuhahn et al., in revision
COX-expression in human HCC-cells

cirrhosis  tumor  liver  Hep-3B  HuH-7  Hep G2  SK-hep-1  T47D

COX-1  COX-2

2.8-  4.4-

18S  28S

kDa

70-  72-  43-

Hep 3B  HuH-7  Hep G2  SK-hep-1  T47D

COX-1  COX-2  actin
Reduced number of HCC-cells after COX-inhibition

Kern et al., Hepatology 2002
Antiproliferative effect of COX-2 inhibition in HCC-cells

untreated Celecoxib [50μM]

Kern et al., Hepatology 2002
COX-2 inhibitor induced apoptosis

Celecoxib treatment

Kern et al., Hepatology 2002
Selective COX-2 inhibition induces mitochondria-dependent apoptosis.

Kern et al., Hepatology 2002
associated with caspase-8 activation
Reduced HCC growth by COX-2 inhibition (prevention)

Kern et al., Carcinogenesis 2004
Increased apoptosis and reduced proliferation after COX-2 inhibition *in vivo*

Kern et al., Carcinogenesis 2004
Combination treatment of HCC-cells (day 4 after inhibition)

Combination treatment: Celecoxib/IGF-II siRNA

Combination treatment: Cisplatin/IGF-II siRNA

* * *  (super)additive effects

n.s.  no effects
Therapeutic targets?

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<th>Target</th>
<th>Frequency</th>
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<td>COX-2 inhibitory NSARs</td>
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<tr>
<td>IGF-II</td>
<td>20-30%</td>
<td>IGF-IR-inhibitors (e.g. Cyclolignans) Trichostatin &amp; analogs, IFNγ</td>
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<tr>
<td>Ep-CAM</td>
<td>15%</td>
<td>therapeutic hum mAb</td>
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<tr>
<td>SIAH-1</td>
<td>high (70-80%)</td>
<td>Proteasome inhibitors (e.g. MG132)</td>
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Conclusion

• Morphology and staging are important for establishing diagnosis and extent of HCC; they have limited relevance for prognostic and therapeutic assessment

• Novel discriminative markers and therapeutic target structures are needed; high-throughput, unbiased screening techniques (CGH, cDNA- and tissue-microarrays) facilitate their identification

• Identification of novel targets (e.g. IGF-II, COX-2) may allow individualized systemic therapeutic approaches in analogy to established schemes in other malignancies
Role of Pathology

Current practice
- Histomorphological analysis of biopsy and resection specimen

Experimental
- Identification and functional evaluation of target structures and markers

Future
- Predictive pathology (pretherapeutic evaluation, trials)
Profiling

Targets
**IGF-II:** Z. Nong, P. Lund, T. Nussbaum, M. Farsad, K. Breuhahn

**COX-2:** M. Kern, A. Haugg, D. Sahi, M. Schöneweiß, I. Moll, D. Schubert

**ZMMK**
U. Protzer, J. Schulze

**Institut für Pathologie, Köln**
M. Odenthal, V. Dries, H. U. Kasper, H.P. Dienes

**HCC-Konsortium**
(Köln, Bonn, Essen, Hannover)

**VARI, Grand Rapids, MI**
G.F. Vande Woude, B. Haab, R. Haddad

**DKFZ, Uni Heidelberg**
P. Lichter, B. Radlwimmer, M. Müller-Schilling, P. Krammer

T. Ekström ([Karolinska Institut](#))
T. Pietsch ([Neuropath., Uni Bonn](#))

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