Etiology and Pathogenesis of Cholangiocarcinoma

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Cholangiocarcinoma (CCA)

- Definition / Classification
- Epidemiology
- Risk factors
- Pathogenesis
CCA

Definition

Malignant tumor arising from cholangiocytes
the epithelial cells that line the bile ducts

Well- to moderately-differentiated adenocarcinoma
CCA

Epidemiology

- Rare tumor - 5,000 cases / year (US)
- Male to Female ration - 1.3 : 1
- ~70% of intra-hepatic CCA > 65 y.o.
- Several risk factors reported
- Majority of CCA have no identifiable risk factors
- High mortality
Incidence of CCA in US

Period 1975 - 1999

Shaib YH et al., Sem Liv Dis 24;1353:2004
Incidence of Intra-hepatic CCA - WHO

Khan SA et al., J Hepatol 37;806:2002
Death Rates from Intra-hepatic CCA in US

Deaths / 100,000

Years

1973 '74 '75 '76 '77 '78 '79 '80 '81 '82 '83 '84 '85 '86 '87 '88 '89 '90 '91 '92 '93 '94 '95 '96 '97

Patel T Hepatology 33;1353;2001
Survival of Intra-hepatic CCA in US

Time after diagnosis (months)

Probability of survival

- 1980-1984
- 1995-1999
Risk Factors of CCA

- Primary Sclerosing Cholangitis (PSC)
- Liver fluke infestation
  - *Opisthonorchis viverrini*
  - *Clonorchis sinensis*
- Liver cirrhosis
- Hepatitis C
- Caroli’s disease
- Choledochal cysts
- Bile duct adenoma, biliary papillomatosis
Risk Factors of CCA

- Chronic intra-ductal stones
- Surgical biliary-enteric drainage procedures
- Chemicals
  - thorotrast
  - dioxin
  - vinyl chloride
- HIV
- Smoking
- Diabetes mellitus
Pathogenesis of CCA

• Common feature of several CCA risk factors
  Chronic biliary inflammation

• Extra-hepatic vs. Intra-hepatic
  likely different causes/pathogenesis
  origin of tissue - experiments

• Somatic cell alterations and genetic predisposition
  in addition to environmental exposures

• Multiple molecular pathways
PSC and CCA

Reported relative risk of CCA in PSC >1500

PSC - a model disease for CCA development
Cumulative Incidence of CCA in PSC

Years since PSC diagnosis

Incidence of CCA (%)
Molecular Alterations in CCA

**Escape from Senescence**
- p16\(^{INK4}\)
- p53
- p21/WAF1
- Mdm-2
- Telomerase

**Evasion of Apoptosis**
- FLIP
- NO
- Bcl-2
- Bcl-X\(_L\)
- Mcl-1

**Tissue Invasiveness and Metastasis**
- E-cadherin
- α-catenin and β-catenin
- Matrix metalloproteinase
- Human aspartyl ̃ hydroxylase expression
- WISP1v
- VEGF

**Autologous Proliferation Signaling**
- IL-6; gp80/gp130
- HGF/c-met
- EGF/c-erbB-2
- COX-2
- k-ras
Bile Duct Inflammation and CCA

- Reactive oxygen species
- Cytokines (IL-6, TNF-a, etc)
- Inflammatory Cells: T-cells, Macrophages
- NO (nitric oxide)
Hypothesis

Inflammation

↓

iNOS
(inducible nitric oxide synthase)

↓

NO

↓

Carcinogenesis
Pathogenesis of CCA

NO in Carcinogenesis

Modulation of transcription factors

Oncogene expression

Dysregulation of apoptosis

Oxidative DNA Damage

Angiogenesis

Inhibition of DNA repair enzymes
PSC and CCA

**Question**
Does the biliary epithelia in PSC and CCA express iNOS?

**Approach**
iNOS immuno-histochemistry

Liver specimens
- Normal (n=30)
- PSC (n=30)
- CCA (n=20)

Jaiswal M et al., Cancer Res 60;184:2000
Human Biliary Epithelia in PSC and CCA Uniformly Express iNOS

Normal

\[ \text{iNOS positive 0/30} \]

PSC

\[ \text{iNOS positive 30/30} \]

CCA

\[ \text{iNOS positive 20/20} \]

Jaiswal M et al., Cancer Res 60;184:2000
PSC and CCA

**Question**
Is iNOS expression in PSC and CCA associated with oxidatively damaged DNA?

**Approach**
8-oxo-deoxyguanosine (8-oxo-dG)
Immuno-histochemistry
Liver Specimens

- Normal liver (n=10)
- PSC (n=10)
- CCA (n=10)

Jaiswal M et al., Cancer Res 60;184:2000
Oxidative DNA Lesion

Deoxyguanosine (dG) → 8-oxo-deoxyguanosine (8-oxo-dG)

Oxidative Stress

ROS
iNOS Expression in PSC and CCA is Associated with Oxidatively Damaged DNA

Normal

PSC

CCA

8-oxodG positive: 0/10

8-oxodG Positive: 10/10

8-oxodG Positive: 10/10

Jaiswal et al., Gastroenterology 120;190:2001
Oxidative DNA Damage is not Repaired in the Presence of Cytokine Stimulated iNOS Expression with NO Generation.
NO Inhibits Base Excision Repair of Oxidatively Damaged DNA Lesions Promoting Carcinogenesis
Oxidative DNA damage causing point mutations and CCA

iNOS 

Cholangiocytes → NO → Oxidative DNA damage → Gene targets? → CCA

p16, a Tumor Suppressor Gene, Is Inactivated in PSC-associated CCA

p16 Inactivation

- Promoter methylation in CpG islands
- Loss of heterozygosity
- Homozygous deletion
- Point mutations
PSC, CCA and p16

Question
Are p16 point mutations present in PSC cholangiocytes and PSC-associated CCA?

Approach
Patients
- PSC (n=10)
- PSC with CCA (n=10)
- Control (n=10)

Laser Capture Micro-dissection
p16 sequencing
- PCR

p16 Point Mutations in PSC-cholangiocytes

Patients: 5/10, 1/10, None, None

p16 Point Mutations in PSC-associated CCA

Patients  8/10  3/10  None  None

Promoter/Enhancer  Exon 1  Exon 2  Exon 3

No p16 mutations were observed in:

- Hepatocytes
  - PSC
  - PSC with CCA
  - Controls

- Cholangiocytes
  - Controls
p16 Mutations and CCA

Question
What is the functional significance of the p16 promoter point mutations?

Approach
Site directed mutagenesis of the promoter
- 8 point mutations identified in PSC cholangiocytes
Reporter gene assay
- transfected HuH-7 cells
Immuno-histochemistry for p16 protein

p16 Reporter Gene Assay

No mutation
-571 T → C
-493 A → T
-437 G → A
-336 G → A
-317 G → A
-301 G → A
-32 G → A
-8 G → A
pGL2

Relative Luciferase Activity (%)
Immuno-histochemistry for p16 Protein in PSC Cholangiocytes

No mutation

With promoter mutation
(-301 G to A, <50% reduction)

p16 point mutations occur in PSC cholangiocytes and in PSC-associated CCA

- Antioxidants
- iNOS inhibitors

Cholangiocytes $\xrightarrow{\text{NO}}$ Oxidative DNA damage $\xrightarrow{\text{p16 point mutations}}$ gene inactivation $\xrightarrow{}$ CCA
Model for iNOS Mediated COX-2 and Notch-1 Induction in Carcinogenesis

Inflammation

iNOS

NO

COX-2 inhibitor

p38 MAPK inhibitor
JNK inhibitor

JNK inhibitor

COX-2

Notch-1

c-Notch-1

γ-secretase inhibitor

Nuclear Translocation

PGE₂

Hes-1

Cancer phenotype

Cell Proliferation
Anti-apoptosis
Angiogenesis
Tumor migration

Molecular Alterations in CCA

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Conclusions

- Chronic biliary inflammation is important in inducing signaling pathways that contribute to cholangio-carcinogenesis

- Better understanding of these pathways is needed before we can use pharmacological inhibitors for the prevention and treatment of CCA
Summary

- Point mutations occur frequently in the p16 promoter region in PSC cholangiocytes and CCA cells complicating PSC

- Many of these mutations result in reduced promoter activity of p16

Cholangiocarcinoma (CCA)
p53 pathways

CDK4 \( \rightarrow \) cyc D

p21

CDK4 \( \rightarrow \) cyc D

p16

Rb \( \rightarrow \) E2F

Rb \( \rightarrow \) P

E2F

Apoptosis

Cell Cycle

Bax \( \rightarrow \) Bcl-2

Bcl-2