Pathogenesis of Liver Fluke Induced Cancer in Thailand

ICIDR-NIH Grant
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Investigators

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  Andrew Boyd

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  Smarn Tesana
  Thewarach Laha
  Radiology: Eim-orn Mairiang
  Surgery: V. Bhudhisawasdi

  Faculty of Public Health
  Bandit Thinkhamrop
Liver Fluke & Cholangiocarcinoma

- Highest incidence
- Northeast Thailand
Incidence of cancer (male)


Khon Kaen

- Liver: 85.0
- Lung: 19.9
- Colon & rectum: 7.1
- Skin: 4.8
- Leukaemia: 6.1
- Oral cavity & pharynx: 4.2
- Non-Hodgkin lymphoma: 4.2
- Bladder: 3.0
- Brain: 3.2
- Stomach: 4.9

Bangkok

- Liver: 25.6
- Lung: 16.6
- Colon & rectum: 14.4
- Skin: 5.0
- Leukaemia: 5.2
- Oral cavity & pharynx: 6.6
- Non-Hodgkin lymphoma: 5.0
- Bladder: 4.9
- Brain: 3.1
- Stomach: 4.9

Chiang Mai

- Liver: 14.6
- Lung: 27.6
- Colon & rectum: 8.8
- Skin: 3.1
- Leukaemia: 5.9
- Oral cavity & pharynx: 6.4
- Non-Hodgkin lymphoma: 5.9
- Bladder: 4.6
- Brain: 4.5
- Stomach: 4.9

Songkla

- Liver: 5.7
- Lung: 13.6
- Colon & rectum: 7.2
- Skin: 4.0
- Leukaemia: 3.1
- Oral cavity & pharynx: 12.9
- Non-Hodgkin lymphoma: 4.6
- Bladder: 3.6
- Brain: 3.6
- Stomach: 4.6
Incidence of CCA (per 100,000) and prevalence of OV infection in Khon Kaen
(Data from Sriamporn et al., 2004)

CCA incidence = 188.8 (93.8-317.6) per 100,000
OV prevalence 24.5%
## Risk factors of Cholangiocarcinoma: community-based study

<table>
<thead>
<tr>
<th>Risk factors</th>
<th>n</th>
<th>Adjusted odd ratio</th>
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<tbody>
<tr>
<td><strong>OV egg count</strong></td>
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<tr>
<td>0</td>
<td>410</td>
<td>1</td>
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<tr>
<td>1-1500</td>
<td>753</td>
<td>1.67 (0.2-16.3)</td>
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<tr>
<td>1500-6000</td>
<td>477</td>
<td>3.23 (0.4-29.5)</td>
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<tr>
<td>&gt; 6000</td>
<td>167</td>
<td>14.08 (1.67-118.6)</td>
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<tr>
<td><strong>Antibody to OV</strong></td>
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<td>OD&lt;0.2</td>
<td>180</td>
<td>1</td>
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<tr>
<td>OD&gt;0.2</td>
<td>73</td>
<td>27.09 (6.3-116.57)</td>
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<tr>
<td><strong>Sex</strong></td>
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<tr>
<td>Female</td>
<td>950</td>
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<tr>
<td>Male</td>
<td>857</td>
<td>3.0 (0.8-11.2)</td>
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<td><strong>Age</strong></td>
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<td>&gt;50</td>
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<td><strong>Praziquantel treatment</strong></td>
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<td>1</td>
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<td>3.4</td>
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<td>2-4</td>
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(Haswell-Elkins et al, 1994; Chuenroongroj, 2000; Honjo et al., 2005)
Comparative number of cases in 10 consecutive years (1995-2004) of 10 leading sites of cancer in both sexes.

Tumor Registry, Srimagarind Hospital-KKU (2004)
Liver fluke

Carcinogens

Other factors
Liver flukes

Opisthorchis viverrini  Opisthorchis felineus  Clonorchis sinensis
Life cycle of *Opisthorchis viverrini*
Bithynia snails

Cyprinoids fish

Metaceria in muscle
Pathology of opisthorchiasis

- Inflammation
- Epithelial desquamation
- Epithelial hyperplasia
- Goblet cell metaplasia
- Periductal fibrosis/cholangiofibrosis
- Granulomatous inflammation
- Adenomatous hyperplasia

(Sripa, 2003)
Immunopathology

(Sripa & Kaewkes, 2000)
Nitric Oxide (NO)

Inflammation
- Macrophage
- Eosinophil
- Epithelium
  - iNOS
  - IFN, TNF
  - T cells

Immunosuppression
- (37 kDa OV antigen)
- Inhibit DNA repair (BER-inflammation)
- 8-oxodG (G:C-T:A)

Genotoxic
- NO & metabolites
- 8-oxodG (G:C-T:A)
- DNA damage
- amines
- NDMA
- CYP
- adduct
8-oxodG – an oxidative DNA damage marker
Apoptosis (TUNEL)
Epithelial hyperplasia

BrdU labeling
Liver fluke infection

Physical

Epithelial desquamation
Parasite molecules

Epithelial hyperplasia
Goblet cell metaplasia
Adenomatous hyperplasia

DNA damage

Immunopathology

iNOS

ROI

Endogenous N.

Exogenous N. (dietary)

Inflammation

macrophages, mast cells, eosinophils, lymphocytes

Periductal fibrosis

Bile stasis
Ascending cholangitis

Genetic alterations

Malignant transformation

Cholangiocarcinoma
Research Plan

- **AIM 1**: The hypothesis is that *O. viverrini* (OV) secretes carcinogenic molecules. To address this hypothesis, the study involves identification of potentially carcinogenic molecules from the excretory/secretory (ES) products of *O. viverrini* by
  - Characterize the transcriptome of adult OV using an expressed sequence tag (EST) approach.
  - Characterize the secretome of adult OV using proteomics.
  - Identifying carcinogenic moieties from ES products of *O. viverrini* taking advantage of human and animal cell lines; proliferation and mutation will be investigated.
**AIM 2:** The hypothesis is that people who develop CCA display a characteristic phenotype of inflammation to *O. viverrini* that predisposes to DNA damage and malignancy. To assess inflammatory parameters, we will

- Access the human population in Khon Kaen, a province in N.E. Thailand that is highly endemic for OV and CCA. Participating human subjects will be assigned to different groups based on intensity of OV infection with groups including (a) uninfected controls, (b) infection, and (c) CCA patients.

- Assess and correlate inflammatory markers including cytokine profiles and serum antibodies with pathology (as assessed by ultrasonography or other diagnostic procedures) and intensity of OV infection.

- Assess parasite burden, pathology and inflammation at the outset of the study. Re-infection rates and inflammatory markers will be determined subsequently on an annual basis for 3 years.
Screening (2000)

Stool examination

OV egg-negative

OV egg-positive

OV-negative = 400
OV-positive = 400

Samples (800)

OV-negative (400)

OV-positive (400)

Ultrasound

Blood

Feces

Antibody (IgG1-4, IgE)

OV-egg

Grading

Cytokines

T-cell proliferation

Inflam markers

Follow up

Yearly (3yrs)

PZQ
Progress report

- **ESTs**
  - cDNA library construction (adult worm)
  - GenBank submission (186 OV-ESTs)
  - 896 clones have been sequenced
  - 4000 more clones will be sequenced

- **Proteomics**
  - ES proteomics
  - Tegumental glycoglycoproteins
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Some recombinant protein expressed

- Cysteine protease
- Aspartic protease
- Thioredoxin peroxidase (TPx)
Similarity of *O. viverrini* cysteine protease

The cDNA sequence of the novel cysteine protease gene show a very close similarity to *C. sinensis, P. westermani*, human and mouse CP, 81%, 57%, 45%, and 44%, respectively.
SDS-PAGE & Immunoblot

SDS-PAGE

Immunoblot

1 = flow through
2 = purified expressed protein

30 kDa
Immunolocalization

Negative control

Vitelline gland

Gut

Seminal receptacle
Effects on cell proliferation

- Transwell (Costar)
- Culture OV ontop biliary cells on polycarbonate membrane.
- Culture biliary cells on well and OV on polycarbonate membrane
Effects of ES and CP on biliary cells

KKU-M214 cells/OV-ES Antigen

KKU-M214 cells/CP1 Antigen
Effects on cell morphology

Biliary cells

Effects of OV recombinant cysteine protease on KKU-100 cells
Acknowledgments

Institutional support
Faculty of Medicine – KKU
Liver Fluke and Cholangiocarcinoma Research Center

Graduate Students
Kantima Ninlawan
Krajang Talabnin
Sirikachorn Tangkawatana
Sutas Suttiprapa
Natthawut Kaewpitoon
Porntip Pinlaor
Michael Smout

Research Assistance
Manop Sripa
Apa Surapaitoon
Nonglack Kaewkai
Suwit Balthaisong

Grant support
ICIDR-NIH
The Thailand Research Fund (TRF)
Thailand-Tropical Disease Research (T-2)
RGJ-PhD Program

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