The liver fluke *Opisthorchis felineus*: molecular biology and carcinogenic potential

Viatcheslav A. Mordvinov  
The Federal Research Center Institute of Cytology and Genetics  
Siberian Branch of the Russian Academy of Sciences  
Novosibirsk, Russia  
E-mail: mordvin@bionet.nsc.ru
Epidemiologically important fish-borne liver trematodes

- Family Opisthorchiidae: *Opisthorchis felineus* (Rivolta, 1884), *O. viverrini* (Poirier, 1886), and *Clonorchis sinensis* (Loos, 1907)

- These liver flukes are known to cause serious human diseases affecting bile ducts and the gall bladder – opisthorchiasis and clonorchiasis

- According to the Food and Agriculture Organization and World Health Organization, liver flukes family Opisthorchiidae are the 8th in the overall global list of 24 food-borne parasites

- Liver fluke infection is recognized as the major risk factor of cholangiocarcinoma

> An estimated 12.5, 67.3 and 601 million people are currently at risk for infection with *O. felineus, O. viverrini* and *C. sinensis*, respectively
Geographical range of Opisthorchiidae liver flukes

- *Clonorchis sinensis*
- *Opisthorchis felineus*
- *Opisthorchis viverrini*
**Brief history of *Opisthorchis felineus***

<table>
<thead>
<tr>
<th>Year</th>
<th>Event</th>
</tr>
</thead>
<tbody>
<tr>
<td>1884</td>
<td>S. Rivolta, an Italian scientist, described <em>Distomum felineum</em> (sin. <em>Opisthorchis felineus</em>), a new helminth species parasitizing the bile ducts of cat's liver</td>
</tr>
<tr>
<td>1891</td>
<td>In humans, this helminth species was discovered by K. Vinogradov, a professor of the Tomsk University (Russia)</td>
</tr>
<tr>
<td>1895</td>
<td>R. Blanchard determined the taxonomic position of <em>O. felineus</em></td>
</tr>
<tr>
<td>1900-1904</td>
<td>M. Askanazy published information about parasite infection caused by <em>O. felineus</em> at cats and dogs in Italy, France, Holland, Germany and Russia.</td>
</tr>
<tr>
<td>1919-1959</td>
<td>Academician K. Skryabin organized system work on identification and the description of the helminthiasis loci in the territory of the USSR, gave the description of the Opisthorchiasis loci, created the term &quot;biohelminthiasis&quot;</td>
</tr>
</tbody>
</table>
Foci of opisthorchiasis in former USSR

1 - The basin of the Baltic Sea, the rivers Neman and Zapadnaya Dvina
2 - The basin of the Black Sea, the rivers Dnepr, Don and Dnestr
3 - The basin of the Caspian Sea, the rivers Volga and Ural
4 - The Ob–Irtysh basin (The Arctic Basin), the rivers Ob and Irtysh
5 - The Arctic Basin, the river Birjusa
Opisthorchis felineus: life cycle stages

1. Embryonated eggs passed in feces.
2. Eggs are ingested by the snail.
3. Metacercariae in flesh or skin of fresh water fish are ingested by human host.
4. Infective Stage
5. Excyst in duodenum
6. Adults in biliary duct
7. Infective Stage
8. Diagnostic Stage
Infected fish is the only source of *Opisthorchis felineus* infection

- **Dace** (*Leuciscus leuciscus*)
- **Tench** (*Tinca tinca*)
- **Ide** (*Leuciscus idus*)
- **Roach** (*Rutilus rutilus*)
- **Spelding (dried fish)**
- **Frozen sliced raw fish ("stroganina")**
The liver fluke *Opisthorchis felineus*: the parasite presenting a serious public health threat in Western Siberia

Official statistical data (cases/100000)

Published data (Russian medical journals)

Prevalence (%)
- OMSK: 20,8 ± 3,7% (1997 – 1998 years)
- HMAO: 19,6 ± 1,4% (2006 – 2008 years)
- HMAO: 16,3% (1966 – 1975 years)

Autopsy
- TYUMEN: 46% (1966 – 1987 years)

Intensity of *O. felineus* infection

- Mild (<50): 42%
- Moderate (<100): 25%
- Heavy (>1000): 33%
Laboratory of Molecular Mechanisms of Pathological Processes
ICG SB RAS

The main lines of investigations

• Comparative studies of molecular biology of epidemiologically important liver flukes
• Molecular mechanisms of pathogenesis of liver fluke infection

Plan of presentation

• Functional genomics of Opisthorchiidae liver flukes
• Carcinogenic potential of liver fluke *Opisthorchis felineus*
• Search of potential molecular targets for new anthelmintic agents and combinatorial treatment of liver fluke infection
**Opisthorchis felineus** Genome Project

*O. viverrini* and *C. sinensis* but not *O. felineus* have been recently characterized at the levels of genome and transcriptome. To address this knowledge gap, we have sequenced the *O. felineus* genome and used the *de novo* assembled draft genome to gain new insights into genetic features of the liver flukes.

Opisthorchis felineus genome sequencing

<table>
<thead>
<tr>
<th>Type</th>
<th>Insert size</th>
<th>Reads, $10^6$</th>
<th>Coverage to assembly</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>reads</td>
</tr>
<tr>
<td>PE</td>
<td>180 bp</td>
<td>564,0</td>
<td>71</td>
</tr>
<tr>
<td>PE</td>
<td>260 bp</td>
<td>266,7</td>
<td>31</td>
</tr>
<tr>
<td>MP</td>
<td>2 Kbp</td>
<td>12,4</td>
<td>2.0</td>
</tr>
<tr>
<td>MP</td>
<td>4 Kbp</td>
<td>16,0</td>
<td>2.5</td>
</tr>
<tr>
<td>MP</td>
<td>6 Kbp</td>
<td>14,9</td>
<td>2.3</td>
</tr>
<tr>
<td>MP</td>
<td>8,5 Kbp</td>
<td>10,6</td>
<td>1.6</td>
</tr>
</tbody>
</table>

Illumina HiSeq 1500

Computing cluster
Characteristics of genomes: five species of trematodes

<table>
<thead>
<tr>
<th></th>
<th>Genome sizes</th>
<th>Number of genes</th>
<th>Repetitive elements in genome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>O. felineus</strong></td>
<td>680.0 Mbp</td>
<td>11 317</td>
<td>30.3%</td>
</tr>
<tr>
<td><strong>C. sinensis</strong></td>
<td>516 Mbp</td>
<td>16 000</td>
<td>29.6%</td>
</tr>
<tr>
<td><strong>O. viverrini</strong></td>
<td>634.5 Mbp</td>
<td>16 379</td>
<td>30.9%</td>
</tr>
<tr>
<td><strong>S. mansoni</strong></td>
<td>364.5 Mbp</td>
<td>11 809</td>
<td>40%</td>
</tr>
<tr>
<td><strong>F. hepatica</strong></td>
<td>1.3 Gb</td>
<td>11 700</td>
<td>54.2%</td>
</tr>
</tbody>
</table>
Repetitive elements in *O. felineus, C. sinensis, O. viverrini* and *F. hepatica* genomes

The most part (90.1%) of the repeats in *O. felineus* genome are retrotransposons (LTR, LINE and SINE elements), while remaining 9.9% are comprised by DNA transposons. Overall repeat landscape of *O. felineus* genome correspond to *C. sinensis* and *O. viverrini* landscapes.
Genome synteny *O. felineus*, *O. viverrini* and *Clonorchis sinensis*

<table>
<thead>
<tr>
<th>Genome alignment statistics</th>
<th>Coverage</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>O. felineus</em> vs <em>C. sinensis</em></td>
<td>493213134 (0.42)</td>
</tr>
<tr>
<td><em>O. felineus</em> vs <em>O. viverrini</em></td>
<td>161445964 (0.26)</td>
</tr>
</tbody>
</table>

Alignment of the top 10 *O. felineus* contigs with the highest coverage by *O. viverrini* and *C. sinensis* genomic sequences

The *O. felineus* contig sequence is represented by middle grey bar. Alignment of the *C. sinensis* (CS) genomic sequences is shown by upper bar, alignment of the *O. viverrini* (OV) genomic sequences is shown by lower bar. Aligned sequences from the same contig have the same color.
Considerable variation in the liver fluke genomes

Structural similarity between the *O. felineus* and *C. sinensis* genomes is higher as compared with that of *O. viverrini* to *O. felineus* and to *C. sinensis*

These data match well the results of chromosome analysis: *O. felineus* and *C. sinensis* have seven pairs of chromosomes versus *O. viverrini* carrying six chromosome pairs

Genome-wide synteny between *O. felineus*, *O. viverrini*, and *C. sinensis*
Phylogenetic relationships

- *C. sinensis* and *O. viverrini* diverged almost immediately after *O. felineus* separated from the common ancestor of these three liver fluke species.

- A comparison of the phylogenetic trees for the three studied opisthorchiids and the data on their synteny suggests that the *O. viverrini* genome was structurally remodeled after it had diverged from its common ancestor with *C. sinensis*.

- Taken together, results of analysis of the synteny between three opisthorchiid species and of their phylogenetic relationships demonstrate that *O. felineus* and *C. sinensis* are closely related and do not support separation of *C. sinensis* from the genus *Opisthorchis*.

- Presumably, *C. sinensis* occupies an intermediate position between *O. felineus* and *O. viverrini*.
The predicted cDNAs of *O. felineus* were aligned to the *O. viverrini* and *C. sinensis* genomes using Spaln2 splice-aware aligner. The best found alignments were mapped back to the *O. felineus* genome. Only reciprocal-best pairs that cover each other by >90% were retained. The described workflow allowed for identification of nearly-identical 'orthologous' coding sequences for 9952 (87%) and 10077 (88%) genes for the comparisons to *O. viverrini* and *C. sinensis*, respectively.
Differences in gene expression of *O. felineus*, *O. viverrini*, and *C. sinensis*

• Expression of most genes of these three opisthorchiid species is at almost the same level independently of the sources of RNA-seq data, obtained by different laboratories.

• Some genes have a significantly different level of expression. In total, 61 such genes were recorded for the pair *O. viverrini*–*O. felineus* and 160, for *O. felineus*–*C. sinensis*. The genes with expression values differing more than fourfold (p < 0.01) are colored red.

• Products of majority of the differentially expressed genes contain domains characteristic for helminth-secreted proteins.
Ethacrynic acid inhibits the enzymatic activity *O. felineus* GST sigma in a dose-dependent manner

<table>
<thead>
<tr>
<th>Incubation media</th>
<th>Inhibitor</th>
<th>Protein quantity, µg</th>
<th>ΔA340 nm</th>
<th>Inhibitor concentration, µM</th>
<th>Average activity ± SD, u/mg</th>
<th>%, inh</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>25</td>
<td>0.11</td>
<td>0</td>
<td>0.18 ±0.02</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>50</td>
<td>0.21</td>
<td>0</td>
<td>0.19 ±0.03</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>100</td>
<td>0.34</td>
<td>0</td>
<td>0.16 ±0.03</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>25</td>
<td>0.09</td>
<td>6</td>
<td>0.16 ±0.01</td>
<td>9.69</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>25</td>
<td>0.051</td>
<td>10</td>
<td>0.12 ±0.02</td>
<td>36.10 *</td>
<td></td>
</tr>
<tr>
<td>EA</td>
<td>25</td>
<td>0.047</td>
<td>100</td>
<td>0.088 ±0.003</td>
<td>49.18 **</td>
<td></td>
</tr>
</tbody>
</table>

| Worm Lysates  | Inhibitor | Protein quantity, µg | ΔA340 nm | Inhibitor concentration, µM | Average activity, u/mg %, inh |
|---------------|-----------|---------------------|----------|----------------------------|-----------------------------|--------|
| -             | 12.3      | 0.18                | 0        | 1.25 ±0.18                 | 0                           |
| EA            | 12.3      | 0.14                | 20       | 0.80 ±0.10                 | 36.12 *                     |
| EA            | 12.3      | 0.06                | 100      | 0.32 ±0.04                 | 74.1 ***                    |
| EA            | 12.3      | 0.05                | 200      | 0.31 ±0.01                 | 75 **                       |

The reaction is measured by observing the conjugation of 1-chloro, 2,4-dinitrobenzene with reduced glutathione. Ethacrynic acid was used as an inhibitor for GST sigma. *p < 0.05; **p < 0.01; ***p < 0.005. SD: standard deviation; EA: Ethacrynic acid. The calculated IC₅₀ was 60.8 µM.

**O. felineus** Glutathione S-transferase sigma (prostaglandin D synthase)

**mRNA abundance of genes coding for excretory/secretory products**

<table>
<thead>
<tr>
<th>Glutathione Peroxidase</th>
<th>Glutathione S-transferase (GST)</th>
<th>Thioredoxin Peroxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>O. felineus</td>
<td>O. viverrini</td>
<td>C. sinensis</td>
</tr>
</tbody>
</table>

**Immunostaining of GST sigma *O. felineus***

A: Hamster sample: Control
B: Hamster sample: *O. felineus* infection
C: Human sample: *O. felineus* negative
D: Human sample: *O. felineus* positive

E: epithelium of bile ducts; BD: bile ducts. Epithelium cells are indicated with arrows.
Summary

- The draft *O. felineus* genome size is approximately 684 Mbp, being slightly longer as compared with *C. sinensis* and almost the same as the *O. viverrini* genome; and all three genomes have very similar content and diversity of repetitive elements.

- Expression levels of most genes are practically the same in *O. felineus*, *O. viverrini* and *C. sinensis*. This suggests a high similarity of all biological processes in adult liver flukes that colonize the bile ducts of mammals.

- Our data can be used for study of genetic mechanisms underlying a complex life cycle of liver flukes and the adaptation of parasites to environmental factors in different climatic conditions and to different host species.
Carcinogenic potential of liver fluke

Opisthorchis felineus
Liver flukes infection is strongly associated with cholangiocarcinoma

International Agency for Research on Cancer: *O. viverrini* and *C. sinensis* were both classified as “carcinogenic to humans” (Group 1)

www.thelancet.com/oncology Vol 10 April 2009

Carcinogenic potential of *O. felineus* is not studied

Cholangiocarcinoma (CCA)
Worldwide incidence of Cholangiocarcinoma (cases/100,000)

Non rare cancer > 6/100,000
Rare cancer < 6/100,000
Incidence of Liver cancer in Russia (cases/100,000)

Russian Federation 2012
Cancer - 525931 cases
Liver cancer - 6287
Cases/100,000 - 4,6
The 14th position (1,5%)

Tyumen and HMAO 1962-1971 years
Liver cancer - 1225
Cases/100,000 - 9,4

Moscow - 1,7
Tyumen and HMAO – 9,4
Tobolsk – 18,7
Yamal – 8,2
HMAO – 20,7
Tomsk – 7,0

Russian Federation - 4,6 (1997 – 2012 years)

Non rare cancer > 6/100,000 cases
Rare cancer < 6/100,000 cases

Liver and bile duct cancer in Russia

Percentage of liver cancer types

Moscow
- CCA (14%)
- HCC (85%)

Tyumen
- CCA (79%)
- HCC (20%)

Chronic Opisthorchiasis (Tobolsk Mortuary, autopsy data, 1950-1987)

- Pancreatic cancer: 1.70%
- Bile ducts cancer: 1.30%
- Liver cancer: 7.40%
- Cirrhosis: 1.50%
- Purulent complication: 4.50%

Liver fluke infection - more than 10 years
Two-step model of cholangiocarcinogenesis

Scheme of the experiment

I
Control

II
DMN

III
OF

IV
OF+DMN

– infection with *O. felineus* (OF) 50 metacercariaes

– treatment with dimethylnitrosamine (DMN) 12.5 ppm

• – points of experiment

Mesocricetus auratus
The gross appearance of the liver, gallbladder, and extrahepatic bile ducts at 30 weeks post-infection: A, group I (control); B, group II (dimethylnitrosamine [DMN]); C, group III (infection with Opisthorchis felineus); and D, group IV (O. felineus + DMN). The scale bar is 1 cm. The arrow indicates small whitish yellow neoplasms on the liver surface.
## Development of bile duct cancer in a hamster model

<table>
<thead>
<tr>
<th></th>
<th>control</th>
<th>DMN</th>
<th>OF</th>
<th>OF+DMN</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 week</td>
<td><img src="image1.png" alt="Image A" /></td>
<td><img src="image2.png" alt="Image B" /></td>
<td><img src="image3.png" alt="Image C" /></td>
<td><img src="image4.png" alt="Image D" /></td>
</tr>
<tr>
<td>22 week</td>
<td><img src="image5.png" alt="Image E" /></td>
<td><img src="image6.png" alt="Image F" /></td>
<td><img src="image7.png" alt="Image G" /></td>
<td><img src="image8.png" alt="Image H" /></td>
</tr>
<tr>
<td>26 week</td>
<td><img src="image9.png" alt="Image I" /></td>
<td><img src="image10.png" alt="Image J" /></td>
<td><img src="image11.png" alt="Image K" /></td>
<td><img src="image12.png" alt="Image L" /></td>
</tr>
<tr>
<td>30 week</td>
<td><img src="image13.png" alt="Image M" /></td>
<td><img src="image14.png" alt="Image N" /></td>
<td><img src="image15.png" alt="Image O" /></td>
<td><img src="image16.png" alt="Image P" /></td>
</tr>
</tbody>
</table>

### O. felineus + DMN
- CCA in the liver of the hamsters after 18 weeks p.i.
- After 30 weeks p.i., CCA was detected in all animals of this group

Hepatobiliary histopathological features of the hamster liver. Hematoxylin and eosin (H&E) staining, ×100 magnification.

Conclusion

- Two-step model of cholangiocarcinogenesis: *O. felineus* infection promotes formation of CCA in hamster model

Question

- How can one evaluate histopathological changes in hamsters experimentally infected with *O. felineus*?
Granulomatous inflammation

A. Epithelioid granuloma with multinucleated giant cells, lymphocytes, and eosinophils in portal area, occasionally surrounding eggs. Biliary duct obstruction caused the presence of an adult *O. felineus* liver fluke (magnification ×40)

A1. Dashed line defines magnified area. Evidence of egg granulomata identified by white arrows surrounding by inflammatory cells (magnification ×100)

B. Granulomas with multinucleated giant cell (magnification ×100)

B1. Mononuclear and eosinophilic cell infiltration in portal regions and multinucleated giant cells surrounding eggs (arrow) (magnification ×400)

**O. felineus** infection induces Biliary Intraepithelial Neoplasia

A: normal portal unit with bile duct, hepatic arteriole, portal venule, and a clearly defined limiting plate (magn ×200). The smaller or interlobular bile ducts are lined by cuboidal or low columnar epithelium. No evidence of inflammation (H&E staining).

A1: defines magnified area (magnification ×400) of normal portal unit.

B: biliary obstruction caused by the *O. felineus* worm with portal area enlargement (H&E staining, magnification ×100).

B1: dashed line defines magnified area (magnification ×100).

B’ and B’1’: biliary obstruction caused by *O. felineus*. Bile ducts were lined by enlarged nuclei, with pseudo-stratification, hyperchromatism and some loss of polarity, nuclear crowding, mitotic figures and low-to moderate-grade of dysplasia (BilIN).

C: epithelium lining a large intrahepatic bile duct displays flat hyperplasia with dysplastic changes (BilIN1/2).

C1: increased cellularity, modestly increased pseudo-stratification, and nuclear irregularities including variation in size and polarity, cytologic atypia including presence of nucleoli and loss of polarity (BilIN2).
Conclusion

- Biliary Intraepithelial Neoplasia is not cancer, but it is associated with higher risk for developing cancer in future
- It is a precancerous state

Question

- What molecular mechanisms are involved in the development of a precancerous condition induced by *O. felineus*?
Lipid peroxidation byproducts 4-hydroxynonenal and malondialdehyde were upregulated; these changes in general correlate with the dynamics of hepatic histopathological changes.
Inflammation markers are upregulated in the liver of infected hamsters

Expression of CD68 and CD163 demonstrated by immunohistochemistry.

CD68 – marker of monocyte lineage and tissue macrophages.

CD163 – marker of macrophages with alternative activated phenotype

In the liver of uninfected animals, both CD68 and CD163 proteins were hardly noticeable. Cells expressing both proteins CD68 were found in the inflammatory infiltrates, but also in the liver parenchyma of the infected animals. As long as the infection lasted, the number of cells CD163+ grew
Densitometric analysis of CD68, TNF-α, and CD163 protein levels in the liver of uninfected and infected hamsters

There was direct time-dependent elevation of TNF-α ($R = 0.79; p < 0.001$) and CD163 protein levels ($R = 0.58; p = 0.022$)

Oxysterol-like molecules in developmental stages of *O. felineus*
Oxysterols

• Products of oxidation of cholesterol that arise through enzymatic or non-enzymatic processes
• Oxysterols display mutagenic, genotoxic, pro-oxidative and pro-inflammatory properties that can contribute to malignancy
• Associations between oxysterols and the development and progression of cancer of colon, lung, breast and bile ducts have been proposed
Parasite oxysterol-like molecules in biological fluids during infection

Biofluids of *Opisthorchis felineus*-infected hamster

<table>
<thead>
<tr>
<th>Biofluid</th>
<th>Metabolite</th>
<th>(m/z)</th>
<th>(from <em>O. felineus</em>)</th>
<th>(from <em>O. felineus</em> and after metabolism)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bile</td>
<td></td>
<td>339</td>
<td></td>
<td>117, 226, 257, 430, 483, 617, 872, 908</td>
</tr>
<tr>
<td>Sera</td>
<td></td>
<td>259, 325</td>
<td>(from <em>O. felineus</em>)</td>
<td>317, 539, 588, 667</td>
</tr>
<tr>
<td>Urine</td>
<td></td>
<td>125, 166, 188, 231, 292, 322, 387, 467, 523</td>
<td>(from <em>O. felineus</em> and after metabolism)</td>
<td></td>
</tr>
</tbody>
</table>
Potential mechanisms of liver fluke induced carcinogenesis

Liver Flukes

Physical impact

Epithelium desquamation
Parasite molecules

Excretory-secretory products

OXYSTEROLS

ROS
NO

Inflammation

Immunopathology

Cholestasis
Cholangitis

Periductal fibrosis

Exogenous nitrosamines

Endogenous nitrosation
Oxysterols

DNA damage

Biliary intraepithelial Neoplasia

Cholangiocarcinoma

Fixed genetic alterations

Malignant transformation

Exogenous nitrosamines

Oxysterols

Endogenous nitrosation

Potential mechanisms of liver fluke induced carcinogenesis

Modified
Sripa & Pairojkul (2008)
• Search of potential molecular targets for new anthelmintics

• Combinatorial treatment of liver fluke infection
Praziquantel: *in vivo* study

<table>
<thead>
<tr>
<th>PZQ</th>
<th>Dose, mg/kg</th>
<th>Animals</th>
<th>Worms per animal, ±SD</th>
<th>Worms mortality, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>-</td>
<td>0</td>
<td>22</td>
<td>34±12</td>
<td>0</td>
</tr>
<tr>
<td>+</td>
<td>75</td>
<td>17</td>
<td>10±4</td>
<td>70%</td>
</tr>
<tr>
<td>+</td>
<td>400</td>
<td>7</td>
<td>6±4</td>
<td>81%</td>
</tr>
</tbody>
</table>

DAPI staining of *O. felineus* eggs

Control | Praziquantel, 21 days after treatment

After treatment with praziquantel, 20 - 30% of the worms have demonstrated the normal body structure, motility, and state of eggs.
There is only one cytochrome P450 in parasitic flatworms. In addition, there are no any flavin-containing monooxygenases in nucleotide databases of *O. felineus* and other parasitic flatworms.
CYP is differentially expressed throughout the *O. felineus* life cycle

The level of CYP mRNA expression in adults is significantly higher than in other life stages

The level of CYP gene expression in adult worm is comparable to the expression of such housekeeping genes as paramyosin, alfa tubulin and ubiquitin-like protein

Internal RT-PCR controls: paramyosin, alfa tubulin, ubiquitin-like protein, mitochondrial ribosomal protein L14
Spectrum of activity of *O. felineus* CYP

1. **Penthoxyresorufin**
   - Reacts with **mCYP2B**

2. **Benzoxysterorufin**
   - Reacts with **mCYP3A4**

3. **Ethoxyresorufin**
   - Reacts with **mCYP1A1**

4. **Methoxyresorufin**
   - Reacts with **mCYP1B1**

5. **Chlorzoxazone**
   - Reacts with **mCYP2E1**

3D modeling of *O. felineus* CYP450

**O. felineus** CYP activity in the fluke tissue

Pentoxylresorufin $\rightarrow$ CYP2B $\rightarrow$ Resorufin

- Excretory channel
- Ceacum
- Vitellaria

Pentoxylresorufin

Resorufin
CYP gene knockdown and ketoconazole effects on *O. felineus* adults

CYP mRNA expression after knockdown (RT-PCR)

<table>
<thead>
<tr>
<th></th>
<th>1 day</th>
<th>3 days</th>
<th>5 days</th>
<th>6 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>mock</td>
<td>1.2</td>
<td>1.0</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>LUC</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
<td>1.2</td>
</tr>
<tr>
<td>CYP</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
</tbody>
</table>

% of worms with changed phenotype

- **ketoconazole**
- % of worms with changed phenotype

**CYP gene knockdown or ketoconazole**

Control

Experiment

Deformation of the excretory channels (EC)
Survival curves and pentoxyresorufin metabolizing activity in worm tissues under the influence of RNA interference

Decrease of CYP gene level expression and CYP activity led to change of the phenotype and increase in worms death.
Anthelmintic activity of cytochrome P450 inhibitors:
in-vitro effect on the liver fluke *Opisthorchis felineus*

**Structures of the cytochrome P450 inhibitors**

- **BITC**
- **Disulfiram**
- **Clotrimazole**
- **Miconazole**
- **Ketoconazole**
- **4PIM**

**Survival of newly excysted metacercariae**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Adults IC$_{50}$ (µM)</th>
<th>NEM IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Praziquantel</td>
<td>0.47</td>
<td>0.98</td>
</tr>
<tr>
<td>Miconazole</td>
<td>20.05</td>
<td>0.79</td>
</tr>
<tr>
<td>Clotrimazole</td>
<td>18.03</td>
<td>1.25</td>
</tr>
<tr>
<td>Ketoconazole</td>
<td>13.77</td>
<td>16</td>
</tr>
<tr>
<td>BITC</td>
<td>27.2</td>
<td>16</td>
</tr>
<tr>
<td>4PIM</td>
<td>&gt;&gt;100</td>
<td>&gt;&gt;100</td>
</tr>
</tbody>
</table>

Kaplan–Meier survival curves. Inhibitors: 10 µM BITC, 10 µM disulfiram, 10 µM miconazole, 10 µM clotrimazole, 10 µM 4PIM, 40 µM ketoconazole or DMSO.

4PIM, 4-phenyl imidazole; BITC, benzyl isothiocyanate

Disodium salt of glycyrrhizic acid
A novel supramolecular delivery system for Praziquantel

Structural formulas of PZQ and glycyrrhizic acid

Pharmacokinetics of PZQ and its composition with Na₂GA

Table 1. Solubility of PZQ samples and compositions

<table>
<thead>
<tr>
<th>Sample composition, weight ratio*</th>
<th>PZQ solubility in water, g/L, 37°C</th>
<th>Increase in solubility, n times</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original PZQ</td>
<td>0.234</td>
<td>—</td>
</tr>
<tr>
<td>PZQ/Na₂GA 1/5</td>
<td>0.557</td>
<td>2.38</td>
</tr>
<tr>
<td>PZQ/Na₂GA 1/10</td>
<td>0.687</td>
<td>2.94</td>
</tr>
<tr>
<td>PZQ/Na₂GA 1/20</td>
<td>0.817</td>
<td>3.49</td>
</tr>
</tbody>
</table>

In vivo testing of PZQ and PZQ/Na₂GA 1/10

Up to 10-fold increase in the anthelmintic activity of PZQ in the composition as compared to PZQ alone

Acknowledgements

Maria Pakharukova, Nikita Ershov, Galina Maksimova, Damira Avgustinovich - Federal Research Center Institute of Cytology and Genetics, Novosibirsk, Russia

Alexander Dushkin - Institute of Solid State Chemistry and Mechanochemistry, Novosibirsk, Russia; Tatyana Tolstikova - Vorozhtsov Institute of Organic Chemistry, Novosibirsk, Russia

Paul J. Brindley - George Washington University, Washington, USA

José Manuel Correia da Costa, Nuno Vale - University of Porto, Portugal

Banchob Sripa, Thewarach Laha - Khon Kaen University, Khon Kaen, Thailand
Thank you for your attention!
A study of tribendimidine effects in vitro and in vivo on the liver fluke
Opisthorchis felineus

In vitro

Efficiency of tribendimidine (TBN) IC50 = 0.23 μM for newly excysted metacercariae
IC50 = 0.19 μM for adult worms

Efficiency of praziquantel (PZQ) IC50 = 0.98 μM for newly excysted metacercariae
IC50 = 0.47 μM for adult worms

In vivo

Chronic infection TBN at 400 mg/kg - 77.2% worm burden reduction
PZQ at 400 mg/kg - 76% worm burden reduction

CONCLUSION: The differences between worm burden reduction values after PZQ and TBN treatment were not significant, thus TBN was as effective as PZQ against O. felineus liver flukes. Given the broad-spectrum activity of TBN and efficacy against O. felineus, this drug may be a promising candidate for the treatment of opisthorchiasis felinea and other liver fluke infections.
Institute of Cytology and Genetics (ICG)
Siberian Branch of the Russian Academy of Sciences

Permanent staff: 1400
Post-graduate students: 96
Graduate students: 160

http://www.bionet.nsc.ru
Geographical range of *Opisthorchis felineus* and the prevalence of opisthorchiasis
The predicted secondary structure showed high level of similarity with the CYP2 proteins of mammals, especially with human CYP2E1, which was the reason for selecting the structure of 2E1 as a reference for the 3D modeling (Phyre2 multi-template modeling, 6 templates).
Praziquantel is metabolized in the liver with involvement of 2B1 and 3A4 isoforms of cytochrome P450 (CYP2B1 and CYP3A4)

Whether liver fluke *O. felineus* has functionally active CYP(s)?