Expression of angiogenic markers in murine schistosomiasis mansoni

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23/03/2018
1. Schistosomes
   • Facts and figures

2. Angiogenesis in bladder cancer-associated schistosomiasis

3. Angiogenesis in liver chronic disease-associated schistosomiasis
   • Animal models of *S. mansoni*

4. Angiogenesis markers
   • CD31 - Platelet endothelial cell adhesion molecule (PECAM-1) as endothelial marker
   • MVD - Microvessel Density as angiogenesis marker
   • LYVE-1 - Lymphatic vessel endothelial hyaluronan receptor 1 as lymphangiogenesis marker
Schistosomes: Life cycle

1. Infective Stage
2. Sporocysts in snail (successive generations)
3. Miracidia penetrate snail tissue
4. Eggs hatch releasing miracidia
5. Cercariae released by snail into water and free-swimming
6. Cercariae penetrate skin
7. Cercariae lose tails during penetration and become schistosomulae
8. Circulation
9. Migrate to portal blood in liver and mature into adults
10. Paired adult worms migrate to:
    - mesenteric venules of bowel/rectum (laying eggs that circulate to the liver and shed in stools)
    - venous plexus of bladder

S. mansoni
S. japonicum
S. haematobium
Angiogenesis, or the formation of new endothelial sprouts from preexisting post capillary venules, is a well-known characteristic of inflammatory diseases, wound repair and cancer. Accordingly, angiogenesis is a process in which endothelial cells migrate and divide to form new capillaries, providing support for tumor progression and disease.
Accordingly, angiogenesis is a process in which endothelial cells migrate and divide to form new capillaries, providing support for tumor progression and disease.

Anderson et al, APMIS, 2017
Angiogenesis in liver chronic disease-associated schistosomiasis

• Symmers' portal fibrosis (also called periportal fibrosis) is a characteristic hepatic disease described in schistosomiasis.
• Although estimates are not available, Schistosomiasis must still be considered to be the most frequent cause of liver fibrosis worldwide.
• Angiogenesis, the formation of new blood vessels from pre-existing ones, is recognized as a key event in a basic change occurring during repair by granulation tissue.
• This process seems to precede fibrosis formation in most types of chronic liver disease.
Methodological strategy

12 animals

Control

Infected S. mansoni

50 cercaria Tail immersion

Livers formalin fixed, paraffin embedded, sectioned and stained

12 months
Liver pathology and fibrosis in murine schistosomiasis mansoni. (Left non-infected and Right Infected mice)

Botelho et al, Trop Med Int Health, 2017
Fibrosis in murine schistosomiasis mansoni (Left non-infected and Right Infected mice)

Botelho et al, Trop Med Int Health, 2017
**S. mansoni** infection and CD-31 in liver

Botelho et al, Trop Med Int Health, 2017
S. mansoni infection increases MVD in liver

Botelho et al, Trop Med Int Health, 2017
*S. mansoni* infection increases LYVE-1 expression in liver

Botelho et al, Trop Med Int Health, 2017
Conclusions

• *S. mansoni* infection increases angiogenesis (MVD) and lymphangiogenesis (LYVE-1) in the liver.

• Thus, blocking lymph/angiogenesis may represent the appropriate therapeutic target for the treatment of schistosomal liver fibrosis.
Top 10 World’s deadliest animals

If you’re thinking about sharks, snakes and lions...think again!

200 000 people killed per year
Top 10 World’s deadliest animals

The blog of Bill Gates

Mosquito Week

The Deadliest Animal in the World
By Bill Gates
| April 25, 2014
New Diseases
To improve prospects for curbing six newly targeted diseases—ascaris, trichuris, hookworm, schistosomiasis, Buruli ulcer, and Chagas disease—we are investing in research to better understand their transmission patterns and what tools or interventions are needed to fight them.
The most neglected schistosome among schistosomes

Table 1. Number of citations in PubMed over the last five years, 2008–2012.

<table>
<thead>
<tr>
<th>Parasite Species</th>
<th>Approximate Number of Human Cases</th>
<th>Number of PubMed Citations over the Last Five Years</th>
<th>PubMed Citations per Millions of Human Cases</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schistosoma japonicum</td>
<td>1 million</td>
<td>644</td>
<td>644</td>
<td>Steinmann et al. 2006 [1]</td>
</tr>
<tr>
<td>Schistosoma mansoni</td>
<td>54 million^a</td>
<td>1,371</td>
<td>25</td>
<td>Van der Werf et al. 2003 [3]</td>
</tr>
<tr>
<td>Schistosoma haematobium</td>
<td>112 million^a</td>
<td>342</td>
<td>3</td>
<td>Van der Werf et al. 2003 [3]</td>
</tr>
</tbody>
</table>

^a. Sub-Saharan Africa only  
^b. Search conducted on July 14, 2012

1. Group 1 carcinogen responsible for a unique squamous cell carcinoma of the bladder

2. Female Genital Schistosomiasis (FGS) – Infertility?

3. FGS: 3 – 4 times increased risk in acquiring HIV infection
The neglected schistosome

- Absence of available animal models of urogenital schistosomiasis
- Absence of (1) in vitro culture methodologies for developmental stages and (2) Functional Genomic toolkit to address basic biological questions

In 2012 Schistosoma haematobium got into the postgenomic era with S. mansoni and S. japonicum (in 2009)
In vitro culture of *Schistosoma haematobium* developmental stages

Eggs isolated from liver of infected hamsters

Eggs isolated from intestine of infected hamsters

Rinaldi et al., *PLoS NTDs* 2011

Michael Hsieh, MD, PhD
Stirewalt Endowed Director
Principal Investigator of Schistosomiasis Resource Center at BRI
http://www.afbr-bri.com/schistosomiasis/
mhsieh@afbr-bri.com
In vitro culture of *Schistosoma haematobium* developmental stages

Adults obtained by portal perfusion from infected hamsters

Cercariae obtained by shedding infected *Bulinus truncatus* snails

Schistosomules obtained by mechanical transformation of cercariae

Rinaldi et al., *PLoS NTDs* 2011
Animal models of Schistosomiasis associated bladder cancer

Urothelial dysplasia and inflammation induced by *Schistosoma haematobium* total antigen instillation in mice normal urothelium

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*Urologic Oncology* 29 (2011) 809 – 814
Carcinogenic potential of *S. haematobium* eggs

Tumour-like phenotypes in urothelial cells after exposure to antigens from eggs of *Schistosoma haematobium*: An oestrogen–DNA adducts mediated pathway?

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International Journal for Parasitology 43 (2013) 17–26

![Image of normal urothelial cells (HCV 29)]

→ Normal urothelial cells (HCV 29)

Cell proliferation
Apoptosis
Oxidative stress
Genotoxicity

→ Liquid Chromatography Diode Array Detection Electron Spray Ionisation Mass Spectrometry (LC/UV-DAD/ESI-MS) – investigation of oxysterols (oxidized derivatives of cholesterol)
The Hallmarks of Cancer

• Proliferation
• Apoptosis
• Migration
• Invasion
• Metastasis
• Angiogenesis
The Hallmarks of Cancer

- Proliferation
- Apoptosis
- Migration
- Invasion
- Metastasis
- Angiogenesis
- Metabolism
- Immunity
- Genome instability
- Inflammation
Soluble eggs antigens induced tumor-like phenotype in urothelial cells

**Cell proliferation**

<table>
<thead>
<tr>
<th>SEA (µg/ml)</th>
<th>Absorbance (490nm)</th>
</tr>
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<tbody>
<tr>
<td>C</td>
<td>0</td>
</tr>
<tr>
<td>6.25</td>
<td>2</td>
</tr>
<tr>
<td>12.5</td>
<td>2.5</td>
</tr>
<tr>
<td>25</td>
<td>2.5</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
</tr>
</tbody>
</table>

**Genotoxicity**

- **Control**
- **SEA**

**Apoptosis**

- **Control**
- **SEA**

**Oxidative stress**

- **Control**
- **SEA**

Botelho et al. IJP, 2013