Schisto3. **Activation of sinusoidal endothelial cells correlates with fibrosis stage evaluated by ultrasound and it is mediated by hedgehog pathway in human schistosomiasis mansoni**

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Introduction: One of the most remarkable characteristics of schistosomiasis fibrosis is the vascular remodeling. Hedgehog (Hh) pathway regulates fibrogenic liver repair and there is growing evidence that it also regulates angiogenesis and vascular remodeling. Our aim was to evaluate if the hedgehog pathway regulates the vascular remodeling that occurs in schistosomiasis. **Material and Methods:** A total of 28 wedge liver biopsies (Universidade Federal de Minas Gerais) from patients with different grades of schistosomiasis fibrosis staged by ultrasound (WHO protocol; pattern A=3 patients, D=5, Dc=2, Ec=17, F=1) were used in this study. Fragments of three donor livers that were used for split liver transplantation at Duke Hospital were also included as controls. This project was approved by the Ethics Committee of UFMG and Duke University Ethical Board (204-06). Activation of the Hh pathway (Patched and Gli-2) and activation of endothelial cells (CD31) were evaluated by immunohistochemistry and double immunohistochemistry. The number of positive cells was counted in ten 400x fields per patient (Gli-2 and Gli-2/CD31) or quantified using morphometry (Patched). Primary human sinusoidal endothelial cells (SEC) were isolated by elutriation from residual healthy liver tissues of two donor livers that were utilized for split liver transplantation at Duke University Hospital. Cells were incubated with 5μM Cyclopamine (Hh pathway inhibitor) or its inactive analog Tomatidine and total RNA was collected 0h and 48h and analyzed by Real time PCR. **Results:** We found that patients with schistosomiasis mansoni had more Hh activation markers (receptor and target gene Patched and transcription factor Gli-2) than healthy individuals and the number of hedgehog responsive cells correlates with fibrosis ($p<0.00$; $r=0.64$ and $r=0.83$, respectively). Bile ducts, stromal cells and endothelial cells were Hh responsive. We observed that activated SECs (CD31+) accumulated in patients with schistosomiasis and the majority of CD31+ cells were also responsive to Hh (Gli-2+) and this correlates with fibrosis stage by ultrasound ($p<0.001$; $r=0.97$). Even patients with schistosomiasis but without detectable fibrosis by ultrasound had an increase number of CD31/Gli2 positive cells ($p<0.05$). Primary human SEC fresh isolated do not express activation marker CD31 and are not hedgehog responsive but express the hedgehog inhibitor Hhip. When those cells are activated in culture they repress Hhip, express CD31 and become hedgehog responsive and the inhibition of the Hh pathway repress their activation phenotype ($p<0.005$), suggesting that Hh pathway regulates activation of SEC. **Main conclusions:** Activation of sinusoidal endothelial cells is mediated by Hh pathway and correlates with fibrosis stage by ultrasound in human schistosomiasis mansoni. **E-mail:** dealmeida.thiago@gmail.com