2014

LRH1 as a Driving Factor for Cancer Development

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INTRODUCTION
Cancer is a major public health problem worldwide, with colon cancer ranking as the third most common cause of cancer mortality in the United States. There are an estimated 96,830 new cases and 50,310 deaths in 2014 due to colon cancer.[1] However, the molecular mechanism of colon tumorigenesis is poorly understood and the prognosis is very bad due to multiple drug resistance. Therefore, there is urgent need to identify a novel therapeutic target. Recently, LRH, an orphan nuclear receptor, has been identified as a key regulator for intestinal function with implications for common intestinal diseases including colorectal cancer.[2] We hypothesize that LRH may be a central signaling molecule during the progression of colon cancer. To investigate this, we used the lentiviral expression system to establish stable cell lines with over-expressed LRH and examined that LRH can promote cell proliferation with MTT analysis. We will also detect the LRH downstream proteins’ expression level by Western blot analysis.

METHODS AND MATERIALS
LRH-1 overexpressed stable cell lines
Stable liver and pancreatic cancer cells with constitutive expression of LRH-1 were established by using the Lentiviral Expression system (GeneCopoeia). Sk-Hep-1, AspC-1 and Capan-1 cells were infected with lentiviral particles. Twenty-four hours after infection, cells were incubated with corresponding media for 24 h, then treated with 0.8 μg/mL (for Sk-Hep-1), 8 μg/mL (for Capan-1) and 3 μg/mL (for AspC-1) puromycin overnight to eliminate uninfected cells and thus yield mass populations of puromycin-resistant cells expressing the LRH-1. The LRH-1 protein level in stable pancreatic cancer cell lines was confirmed by Western blot.

Western Blot Analysis
Cell lysates were treated with ConA-sepharose beads overnight followed by centrifugation to remove cadherin-bound β-catenin. Total Cell lysates and non-membrane bound cell lysates were separated by SDS-PAGE and transferred to nitrocellulose membranes. Western blot analysis was performed using primary antibodies against LRH1 (Abcam, ab125034), c-Myc (Cell signaling Technology, 85605), cyclin D1 (Santa Cruz, sc-8396), cyclin E1 (Santa Cruz, sc-247), β-catenin (Cell signaling Technology, 89562), calpain I (Santa Cruz, sc-7531), estrogen receptor alpha (Era) (Santa Cruz, sc-7207), PCNA (Santa Cruz, sc-7967). Protein bands were visualized by IRDye® 800RD Infrared Dye and IRDye® 800CW Infrared Dye and exposed on Odyssey image system (LI-COR).

RESULTS
1. Establishment of colon cancer cell line with constitutive LRH-1 overexpression
We did transfection on 3 colon cancer cell lines (CaCo-2, LS-180 and SK-Co-1) with LRH-1 plasmid, and the results showed no or very weak LRH-1 protein expression.

2. LRH-1 upregulates downstream target proteins PCNA (in liver cancer), cyclin D1/E1, ERα and Calpain1 (in pancreatic cancer)
We examined the potential mechanisms of LRH-1 involvement in liver and pancreatic cancer by measuring its downstream target genes. Western blot analysis revealed that PCNA, cyclin D1 and cyclin E1 (full-length [FL]) and truncated T1/T2 isoforms) were upregulated by LRH-1 in stable PC cells. Cyclin E is converted to T1/T2 by calpain1 through post-translational processing. LRH1-mediated cyclin E1/T1/T2 overexpression was attributed to upregulation of ERα-Calpain1 signaling (Fig. 2).

3. LRH-1 promotes liver and pancreatic cancer cell proliferation
LRH-1 overexpression resulted in a significant increase in liver and pancreatic cancer cell proliferation compared with the control (Fig. 3).

4. Tumorigenicity of LRH-1 in vivo
In vitro observations suggest that LRH-1 expression is important for pancreatic cancer cell proliferation. Therefore, we explored its oncogenic role in vivo using a murine subcutaneous (s.c.) model (Fig. 5). We measured the resulting tumor growth in the immune deficient mice after injection of parental and LRH-1-transfected pancreatic cancer cells. Following introduction and expression of LRH-1, Capan-1 cells generated significantly enhanced subcutaneous tumor growth characterized by a significantly increased tumor weight compared to vectors.

FIGURES

Fig. 1: Established cell lines and their growth rate.

Fig. 2: Western Blot analysis of LRH-1 and its downstream target genes in liver cancer and pancreatic cancer cells.

Fig. 3: Growth curves of liver and pancreatic cancer cells with LRH-1 overexpression. Accelerated cell growth was observed in LRH-1 overexpressed Sk-Hep1 (Liver cancer), Capan-1 and AspC-1 (Pancreatic cancer) cells (in contrast, untreated tumor, Student’s t-test, 2-tailed, P<0.05).

Fig. 4: Growth curves of liver and pancreatic cancer cells with LRH-1 overexpression in vivo. The subcutaneous tumor model was established by injection of Capan-1 cells transfected with LRH-1 and Vector control. Compared to vector, LRH-1 overexpression significantly increased tumor volume (Student’s t-test, 2-tailed, P<0.05).

Fig. 5: Western Blot analysis of LRH-1 and its downstream target genes in liver cancer and pancreatic cancer cells.

MITT Assay
Liver and pancreatic cancer cells (vectors vs. LRH1 overexpressing Sk-Hep-1, Capan-1 and AspC-1 (1.7×10⁶ cells per well) were seeded in 24 well plates and cultured for 4 days. Cells were incubated with MITT solution (Sigma-Aldrich) in medium (10% v/v) at 37 °C for 3 h. Then 200μL DMSO was added. Plates were analyzed daily using a plate reader at a wavelength of 570 and 690 nm, respectively. The background absorbance of multi-well plates measured at 690 nm was subtracted from the measurement at 570 nm.

FUTURE WORK
• Establish other stable pancreatic and colon cancer cell lines overexpressing LRH-1 using lentiviral expression system.
• Confirm the expression of LRH-1 by Western blot and RT-PCR.
• Examine the expression levels of cancer stem cell markers.
• Determine if LRH-1 overexpression enhances migration, invasion and sphere formation of pancreatic and colon cancer cells.

REFERENCES

ACKNOWLEDGEMENT
Professor Jack R. Wands
Liver Research Center, Division of Gastroenterology, Warren Alpert Medical School of Brown University