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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](Figure1). 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, LRH1, 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 LRH1 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 LRH1 and examined that LRH1 can promote cell proliferation with MTT analysis. We will also detect the LRH1 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 overtime to eliminate un-infected 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, #5605), cyclin D1 (Santa Cruz, sc-8396), cyclin E1 (Santa Cruz, sc-247), β-catenin (Cell signaling Technology, #9562), calpain1 (Santa Cruz, sc-7531), estrogen receptor alpha (ERα) (Santa Cruz, sc-7207), PCNA (Santa Cruz, sc-7907). Protein bands were visualized by IRDye® 680RD Infrared Dye and IRDye® 800CW Infrared Dye and exposed on Odyssey image system (LI-COR).

MTT Assay

Liver and pancreatic cancer cells (vectors vs. LRH1 overexpressing Sk-Hep-1, Capan-1 and AsPC-1) (1.7×10^4 cells per well) were seeded in 24 well plates and cultured for 4 days. Cells were incubated with MTT 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.

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) [3,4]
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 E 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

	ESTIMATED NEW CASES			ESTIMATED DEATHS		
	BOTH SEXES	MALE	FEMALE	BOTH SEXES	MALE	FEMALE
All sites	**1,665,540**	**855,220**	**810,320**	**585,720**	**310,010**	**275,710**
Oral cavity & pharynx	42,440	30,220	12,220	8,390	5,730	2,660
Tongue	13,590	9,720	3,870	2,150	1,450	700
Mouth	11,920	7,150	4,770	2,070	1,130	940
Pharynx	14,410	11,550	2,860	2,540	1,900	640
Other oral cavity	2,520	1,800	720	1,630	1,250	380
Digestive system	**289,010**	**162,730**	**126,880**	**147,260**	**84,970**	**62,290**
Esophagus	18,170	14,660	3,510	15,450	12,450	3,000
Stomach	22,220	13,730	8,490	10,990	6,720	4,270
Small intestine	9,160	4,880	4,280	1,210	640	570
Colon#	96,830	48,450	48,380	50,310	26,270	24,040
Rectum	40,000	23,380	16,620			
Anus, anal canal, & anorectum	7,210	2,660	4,550	960	370	580
Liver & intrahepatic bile duct	33,190	24,600	8,590	23,000	15,870	7,130
Gallbladder & other biliary	10,650	4,960	5,690	3,630	1,610	2,020
Pancreas	46,420	23,530	22,890	39,590	20,170	19,420
Other digestive organs	5,760	1,880	3,880	2,130	870	1,260
Respiratory system	**242,550**	**130,000**	**112,550**	**163,660**	**90,280**	**73,380**
Larynx	12,630	10,000	2,630	3,610	2,870	740
Lung & bronchus	224,210	116,000	108,210	159,260	86,930	72,330
Other respiratory organs	5,710	4,000	1,710	790	480	310
Bones & joints	**3,020**	**1,680**	**1,340**	**1,460**	**830**	**630**
Soft tissue (including heart)	**12,020**	**6,550**	**5,470**	**4,740**	**2,550**	**2,190**
Skin (excluding basal & squamous)	**81,220**	**46,630**	**34,590**	**12,980**	**8,840**	**4,140**
Melanoma-skin	76,100	43,690	32,210	9,710	6,470	3,240
Other nonepithelial skin	5,120	2,740	2,380	3,270	2,370	900
Breast	**235,030**	**2,360**	**232,670**	**40,430**	**430**	**40,000**
Genital system	**338,450**	**243,460**	**94,990**	**58,970**	**30,180**	**28,790**
Uterine cervix	12,360		12,360	4,020		4,020
Uterine corpus	52,630		52,630	8,590		8,590
Ovary	21,980		21,980	14,270		14,270
Vulva	4,850		4,850	1,030		1,030

Fig.1. Estimated new cancer cases and deaths by sex, United States, 2014

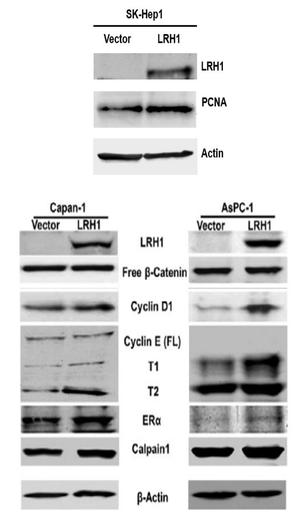

Fig.2. LRH-1 upregulated downstream target genes in liver cancer and pancreatic cancer cells.

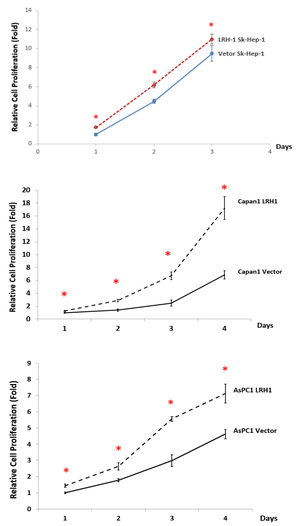

Fig.3. Growth curves of liver and pancreatic cancer cells with LRH-1 overexpression. Accelerated cell growth was observed in LRH-1 overexpressed Sk-Hep-1 (Liver cancer), Capan-1 and AsPC-1(Pancreatic cancer) cells than in controls (Student t test, 2-tailed, *p<0.05).

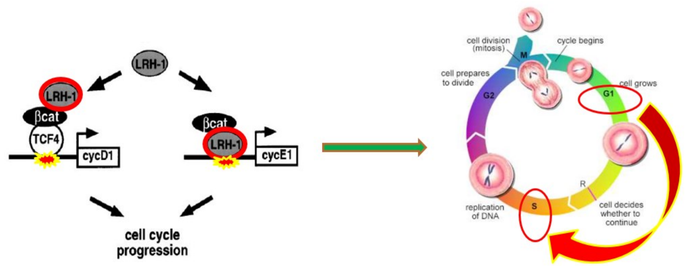

Fig.4. LRH-1 promote cell proliferation by inducing Cyclin D1 and E1 through β-catenin/Tcf-4 signaling pathway.

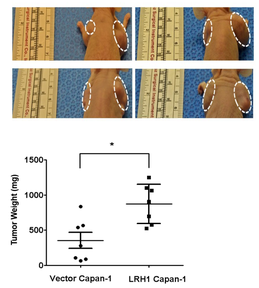

Fig.5. LRH-1 promoted pancreatic tumor growth in nude mice. The subcutaneous tumor model was established by inoculation of Capan-1 cells transfected with LRH-1 and Vector control. Compared to vectors, LRH-1 overexpression cells produced larger size tumors (paired t test, 2 tailed, **p<0.01).

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:
  CD24 / CD44 / ESA / c-MET / ALDH
- Determine if LRH-1 overexpression enhances migration, invasion and sphere formation of pancreatic and colon cancer cells.

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