RESEARCH ARTICLE

Effects of the Hippo Signaling Pathway in Human Gastric Cancer

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Abstract

Background/Aim: The Hippo signaling pathway is a newly discovered and conserved signaling cascade, which regulates organ size control by governing cell proliferation and apoptosis. This study aimed to investigate its effects in human gastric cancer. Methods: Tumor tissues (n=60), adjacent non-tumor tissues (n=60) and normal tissues (n=60) were obtained from the same patients with primary gastric cancer (GC). In addition, 70 samples of chronic atrophic gastritis (CAG) tissues were obtained from patients with intestinal metaplasia (IM) by endoscopic biopsy. Hippo signaling molecules, including Mst1, Lats1, YAP1, TAZ, TEAD1, Oct4 and CDX2, were determined by quantitative polymerase chain reaction (qPCR). Protein expression of Mst1, Lats1, YAP1, TEAD1 and CDX2 was assessed by immunohistochemistry and Western blotting. Results: Mst1, Lats1 and Oct4 mRNA expression showed an increasing tendency from GC tissues to normal gastric tissues, while the mRNA expression of YAP1, TAZ and TEAD1 was up-regulated (all $P<0.01$). Mst1 and Lats1 protein expression presented a similar trend with their mRNA expression. In addition, YAP1 and TEAD1 protein expression in GC was significantly higher than in the other groups (all $P<0.01$). CDX2 mRNA and protein expression in the CAG group were higher than in the other groups (all $P<0.01$). In GC, mRNA expression of Mst1, Lats1, Oct4, YAP1, TAZ, TEAD1 and CDX2 had a close correlation with lymphatic metastasis and tumor TNM stage (all $P<0.01$). Furthermore, protein expression of Mst1, Lats1 ,YAP1, TAZ, TEAD1 and CDX2 had a close correlation between each other ($P<0.05$). Conclusion: The Hippo signaling pathway is involved in the development, progression and metastasis of human gastric cancer. Therefore, manipulation of Hippo signaling molecules may be a potential therapeutic strategy for gastric cancer.

Keywords: Hippo signaling pathway - gastric cancer - chronic atrophic gastritis - intestinal metaplasia

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Introduction

Gastric cancer (GC) is one of the most common malignant diseases and is the second most common cause of cancer-related mortality in China, with an incidence of 0.4 million new cases and 0.3 million deaths annually (Yang, 2006). At present, the wide accepted pattern of GC development is gastritis-metaplasia-dysplasia-carcinoma sequence, proposed by Correa in 1988. The mechanisms of GC development are complicated, involving the alteration of oncogenes and tumor-suppressor genes, which form molecular genetic basis of malignant transformation and tumor progression (Tajima et al., 2006). Until now, lots of signal pathways regulating cell growth and cell apoptosis have been elucidated.

Hippo signal pathway, first identified in Drosophila, is reported to be a tumor-suppressive signal pathway. In Drosophila, the core components of hippo pathway are Hpo, Sav, Mats, Mts, Yki, and Sd, which are also found to be highly conserved in mammals as Mst, WW45, Mob, Lats, YAP/TAZ and TEAD (Zheng et al., 2011). The transcription factor CDX2, a member of the caudal-related homeobox gene family, normally is mainly expressed in small intestinal and colonic epithelia, but not in gastric epithelium (Freund et al., 1998). CDX2 plays an important role in mammalian early intestinal development and the maintenance of intestinal epithelia. It is reported that CDX2 plays the vital role in the process of intestinal metaplasia in the stomach (Barros et al., 2011).

To better understand the mechanisms underlying GC, we compared the expression of Mst1, Lats1, YAP1, TAZ, TEAD1, Oct4 and CDX2 in GC tissue, non-tumor tissues, normal gastric tissues and CAG tissues with IM, both in mRNA and in protein level. Additionally, we analyzed the association among Hippo signaling pathway, CDX2, lymph node metastasis, as well as tumor, nodes, metastasis (TNM) stages, to evaluate the clinical significance of hippo pathway and provide new clues for gastric cancer.

Materials and Methods

Tumor tissues (n=60), adjacent non-tumor tissues

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Table 1. Sequences of Primers for qPCR

	Forward	Reverse
GAPDH	CACCAGGGCTGCTTTTAACTC	TGGAAGATGGTGATGGGATTT
Mst1	AGACCTCCAGGAGATAATCAAAGA	AGATACAGAACCAGCCCCACA
Lats1	TAGAGCAGAGGGCGCGGAAG	CCAACACTCCACCAGTCACAGA
YAP1	TGAACAAACGTCCAGCAAGATAC	CAGCCCCCAAAATGAACAGTAG
TAZ	CTTGGATGTAGCCATGACCTT	TCAATCAAAACCAGGCAATG
TEAD1	AATCCCACCGCCAAAATTGAGC	TACCATACATTTTGCCTTCGTCT
CDX2	AAGTGAAAACCAGGACGAAAGA	GGATGGTGATGTAGCGACTGTA
0ct4	GTATTCAGCCAAACGACCATCT	CATTGTTGTCAGCTTCCTCCAC

(n=60) and normal tissues (n=60) were obtained from the same patients with primary gastric cancer (GC), who received surgical operation at the affiliated hospital of medical college Qingdao university during 2012-2013. All patients were diagnosed with gastric cancer, and none had received prior radiotherapy or chemotherapy before the surgery. Adjacent non-tumor tissues were dissected by at least 3-cm distance and at most 5 cm distance from the tumor edge, and normal tissues, at least 5-cm distance. 70 cases chronic atrophic gastritis (CAG) tissues were obtained from CAG patients with intestinal metaplasia (IM) by endoscopic biopsy during 2012-2013. All these specimens were stored at -80°C immediately after the operation, and were diagnosed by two independent gastroenterologists. All patients had given written informed consent on the use of clinical specimens for medical research.

Immunohistochemistry

Tissue samples were fixed in 10% formalin, embedded in paraffin, and cut into 4μm thick sections. After antigen retrieval and peroxidase blocking, the sections were incubated with rabbit polyclonal antibody against human Mst1 (1:250 dilution), Lats1 (1:250 dilution), YAP1 (1:250 dilution), TEAD1 (1:400 dilution) and CDX2 (1:500 dilution) at 4°C overnight according to the instruction of antibodies. (Though protein expression of MST1, LATS1, YAP1 had been investigated, and relevant article had been published (Wei et al., 2012), we did similar experiments to ensure the integrity of our data and better to analyze the data.) After washed 3 times with PBS, tissue sections were incubated with a horseradish peroxidase (HRP)-conjugated secondary bodies (1:1000 dilution) at 37°C for 10 min. Then we used DAB substrate kit (Invitrogen) to color the sections and counterstained them with hematoxylin. Protein expression was quantified using a visual grading system based on (A) the extent of staining (percentage of positive tumor cells; graded on a scale of 0-4 where 0, none; 1, 1-25%; 2, 26-50%; 3, 51-75%; 4,>75%) and (B) the intensity of staining (graded on a scale of 0-3 where 0, no staining; 1, weak staining; 2, moderate staining; 3, strong staining). According to the product of (A) and (B), the results can be divided into four levels: 0, negative (-); 1-4, weakly positive (+); 5-8, moderately positive (++); 9-12, strongly positive (+++).

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)

We used qRT-PCR to quantitatively determine the mRNA expression level of Hippo signaling molecules. Total RNA were extracted from each tissue according to the manufacture protocol and reverse transcription was carried out according to the instruction of reverse transcription kit. The reaction system of qRT-PCR was as follows: cDNA 2μL, 2×SYBR Premix Ex TaqII10μL, forward primers 2μL, reverse primers 2μL, ddH_2O 4.8μL. Sequences of primers for qRT-PCR described as Table 1 and GAPDH levels were used as internal control. The qRT-PCR was performed with the following conditions: denaturation at 95°C for 30 s, then 95°C for 5 s, 60°C for 30 s, 40 cycles in total. Electrophoretic images of products in qRT-PCR were shown in Figure 1. The results were expressed as mean+SD, and the comparative Ct method ($2^{-\triangle\triangle Ct}$) was used to analyze the data. Three separate experiments were performed for each clone.

Western Blot Analysis

After grinded, frozen tissue samples were lysed in the mixture of the RIPA and PMSF (the ration was 200:1) for 2 hours. Then, Protein lysates were obtained after ultra-centrifugation, and protein concentration was measured by BCA method. 12 μg protein was electrophoresed in a 12% SDS polyacrylamide gel, and separated protein bands were transferred into polyvinylidene fluoride (PVDF) membranes and the membranes were blocked in 5% skim milk power for 1 hours. After blocked, the membranes were probed with polyclonal antibody against human Mst1 (1:6000 dilution), Lats1 (1:6000 dilution), YAP1 (1:8000 dilution), TEAD1 (1:8000 dilution) and CDX2 (1:500 dilution) for 2 hours according to the instructions of antibodies. Then the membranes were washed 3 times for 10 min with PBST. HRP-conjugated secondary antibodies were added at the dilution ratio of 1:10000, and incubated at room temperature for 1 hour. The membranes were washed 3 times for 5min with PBST, then 3 times for 5 min with PBS. At last, the immunoreative bands were visualized using the ECL detection reagents. β-actin was used as the internal control within the same sample on the same membrane. Three separate experiments were performed for each clone.

Statistical analysis

Statistical analysis was performed using SPSS 18.0. One-way analysis of variance was used to analyze the mRNA and protein expression in qRT-PCR and western blot analysis, and also was used in analyzing the correlation of Hippo signaling molecules expression with clinicopathologic features of gastric carcinoma. χ^2 test was use to analyze the protein expression in immunohistochemistry. Additionally, we used

Table 2. mRNA Expression Levels of Hippo Signaling Molecules in qRT-PCR Experiment

	GC	nontumor	CAG	normal	P0	P1	P2	P3	P4	P5	P6
Mst1	0.0229±0.0089	0.0387±0.0060	0.0418±0.0083	0.0749±0.0233	0	0	0	0	0.176	0	0
Lats1	0.0020±0.0005	0.0056±0.0012	0.0060±0.0014	0.0161±0.0195	0	0.04	0.019	0	0.829	0	0
YAP1	0.2632±0.1704	0.0786±0.0182	0.0578±0.0148	0.0223±0.0132	0	0	0	0	0.164	0	0.018
TAZ	0.0175±0.0120	0.0064±0.0023	0.0042±0.0019	0.0019±0.0009	0	0	0	0	0.043	0	0.029
TEAD1	0.0190±0.0093	0.0070±0.0028	0.0054±0.0034	0.0029±0.0029	0	0	0	0	0.101	0	0.009
CDX2	0.0034±0.0010	0.0015±0.0032	0.0194±0.0090	0.0002±0.0002	0	0.043	0	0.001	0	0.159	0
Oct4	0.0019±0.0009	0.0064±0.0019	0.0070±0.0018	0.0220±0.0095	0	0	0	0	0.473	0	0

P0, the difference among the four groups; P1, GC vs nontumor; P2, GC vs CAG; P3, GC vs normal; P4, nontumor vs CAG; P5, nontumor vs normal; P6, CAG vs normal

Table 3. Protein Expression Levels of Hippo Signaling Molecules in Immunohistochemistry

	GC	nontumor	CAG	normal	P1	P2	P3	P4	P5	P6
Mst1	7(11.67%)	24(40.00%)	29(41.43%)	48(80.00%)	0.001	0	0	1	0	0
Lats1	12(20.00%)	29(48.33%)	43(61.43%)	54(90.00%)	0.002	0	0	0.158	0	0
Yap1	49(81.67%)	32(53.33%)	39(55.71%)	14(23.33%)	0.002	0.002	0	0.86	0.001	0
Tead1	51(85.00%)	37(61.67%)	34(48.57%)	4(6.67%)	0.007	0	0	0.159	0	0
CDX2	43(71.67%)	35(58.33%)	62(88.57%)	0(0.00%)	0.18	0	0	0.024	0	0

P1, GC vs nontumor; P2, GC vs CAG; P3, GC vs normal; P4, nontumor vs CAG; P5, nontumor vs normal; P6, CAG vs normal

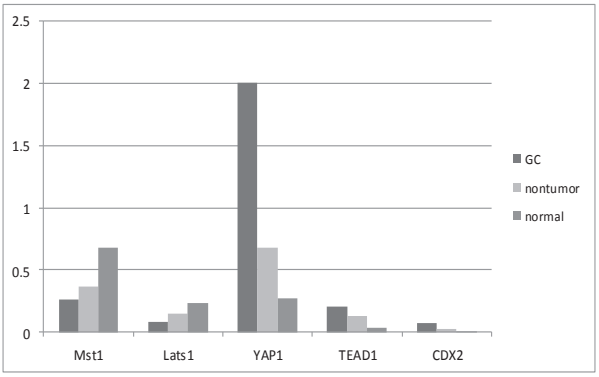

Figure 1. Protein Expression Levels of Mst1, Lats1, YAP1, TEAD1, CDX2 in Western Blot Analysis

Spearman rank association test to analyze the relevance of Hippo signaling molecules expression in GC in immunohistochemistry. *P* value < 0.05 was regarded as statistically significant.

Results

The expression levels of Mst1 and Lats1 in hippo signal pathway

In our experiment, qRT-PCR told us that the mRNA expression levels of Mst1 and Lats1 in GC group dropped obviously compared with the counterparts of non-tumor, CAG and normal groups (P<0.01). And, Mst1, Lats1 mRNA expression showed an increase tendency from gastric cancer tissues to normal gastric tissues (Table 2). Immunohistochemistry experiment told us that Mst1 and Lats1 were predominantly present in the cytoplasm of normal gastric cells, and their protein expression also showed an increase tendency from gastric cancer tissues to normal gastric tissues (Table 3, Figure 2) (These results are consistent with Wen-Chao Wei's discoveries (Wei et al., 2012)). Western blot analysis told us that compared with GC group, Mst1 protein expression in non-tumor and normal group increased 0.42, 1.61 times, respectively (P<0.01). Compared with normal gastric tissues, Lats1

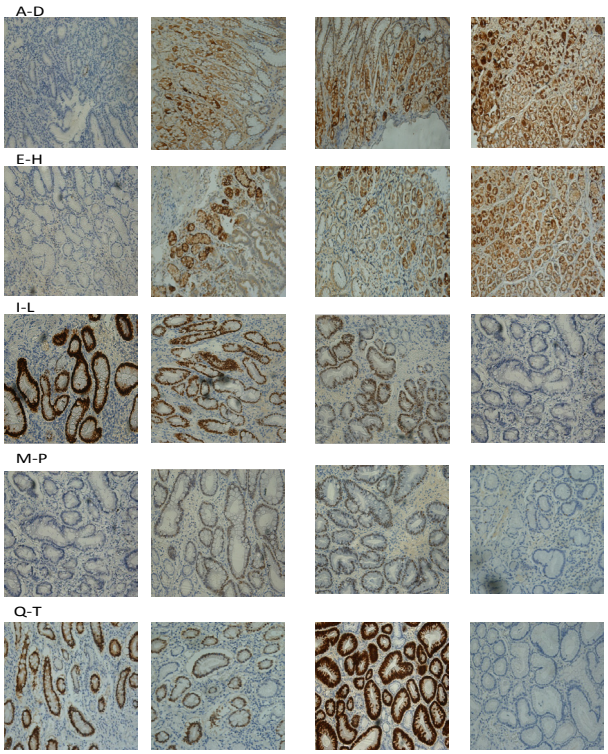

Figure 2. Protein Expression Levels in Immunohistochemistry. Protein extrated from gastric cancer tissues, nontumor tissues, CAG tissues and normal tissues were performed in immunohistochemistry. A-D: Mst1; E-H: Lats1; I-L: YAP1; M-P: TEAD1; Q-T: CDX2 (×200)

protein expression in gastric cancer tissues and non-tumor tissues decreased by 66.78%, 36.96%, respectively (P<0.01), which further confirmed the results in qRT-PCR and immunohistochemistry experiment (Figure 1, 3).

The expression levels of YAP1, TAZ and TEAD1 in hippo signal pathway

Conversely, the mRNA level of YAP1, TAZ and TEAD1 were all significantly elevated in gastric cancer tissues compared with non-tumor, CAG, normal tissues

Table 4. Correlation of Hippo Signaling Molecules Expression with Clinicopathologic Features of Gastric Carcinoma

	Age		Lymphatic metastasis		TNM stage	
	<50	>50	-	+	TNM12	TNM34
MST1	0.0231±0.0074	0.0228±0.0098	0.0281±0.0072	0.0152±0.0048[b]	0.0267±0.0078	0.0147±0.0048[b]
LATS1	0.0021±0.0005	0.0019±0.0006	0.0023±0.0004	0.0015±0.0003[b]	0.0022±0.0005	0.0015±0.0002[b]
YAP1	0.2505±0.1868	0.2705±0.1623	0.1627±0.0499	0.4017±0.1774[b]	0.1758±0.0590	0.4363±0.1847[b]
TAZ	0.0170±0.0121	0.0177±0.0121	0.0110±0.0039	0.0272±0.0135[b]	0.0108±0.0038	0.0319±0.0110[b]
TEAD1	0.0178±0.0090	0.0197±0.0096	0.0139±0.0048	0.0267±0.0092[b]	0.0139±0.0048	0.0301±0.0068[b]
CDX2	0.0038±0.0012	0.0032±0.0008	0.0037±0.0011	0.0030±0.0005[a]	0.0036±0.0011	0.0030±0.0006[a]
Oct4	0.0022±0.0010	0.0017±0.0007[a]	0.0022±0.0007	0.0013±0.0008[b]	0.0022±0.0007	0.0011±0.0005[b]

[a]P<0.05; [b]P>0.01

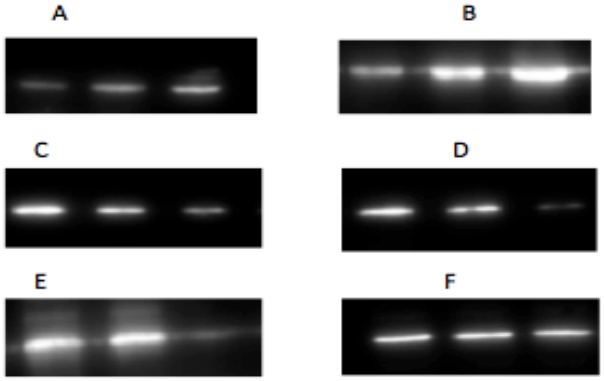

Figure 3. Protein Expression Levels in Western Blot Analysis. Protein extracted from gastric cancer tissues, nontumor tissues and normal gastric tissues were performed in western blot analysis, A: Mst1 ; B: Lats1; C: YAP1; D: TEAD1; E: CDX2; F: β-actin

counterparts (P<0.01) and showed a decrease tendency from gastric cancer to normal tissues (Table 2). Meanwhile, YAP1 and TEAD1 protein were predominantly present in the nuclei of GC cells, and their staining were seldom detected in corresponding non-tumor tissues and chronic atrophic gastritis tissues with intestinal metaplasia, and generally not observed in the nuclei of normal cells (Figure 2) (The results of YAP1 protein expression were consistent with Wen-Chao Wei,s discoveries (Wei et al., 2012)). In our experiment, about 85.00% gastric cancer tissues had detected TEAD1 protein expression, and 61.67%, 48.57%, 6.67% had detected TEAD1 protein expression in non-tumor tissues, CAG tissues and normal tissues, respectively (P<0.01) (Table 3, Figure 2). Western blot analysis further presented that the protein levels of YAP1 and TEAD1 in gastric cancer tissues were approximately 6.35, 4.32 times higher than those in normal gastric tissue (P<0.01) (Figure 1, 3).

The expression levels of CDX2 and Oct4 in hippo signal pathway

More complicatedly, the mRNA expression level of CDX2 was very different from the above molecules. The mRNA level of CDX2 in CAG group was highest, then the GC group and non-tumor group. And in CAG group the mRNA level quantitatively was 4.71 times higher than that in GC group. Surprisingly, there was no expression in the normal gastric mucosa (Table 2). Complicatedly, about 88.57% CAG tissues with IM presented CDX2 protein expression in nuclei, which was only 71.67% in GC tissues, 58.33% in non-tumor tissues and zero in normal gastric tissues (Table 3 and Figure 2). Consistent with the results in immunohistochemistry, there was almost no CDX2 protein expression in normal gastric tissues. And in gastric cancer tissues, CDX2 protein expression is 2.33 times higher than that in non-tumor tissues (Figure 1, 3). Meanwhile, we discovered that compared with GC group, the relative mRNA expression of Oct4 quantity in non-tumor group, CAG group and N group were elevated by 2.37, 2.68, 10.58 times, respectively. And the differences were statistically significant (P<0.01) (Table 2).

The correlation of Hippo signaling molecules expression with clinic-pathological features of gastric carcinoma

In patients, younger than 50 years, the expression of Mst1, Lats1, Oct4 and CDX2 were higher than those in patients older than 50 years, but there were no statistical significances except the expression of Oct4 (P>0.05). However, the expression of YAP1, TAZ, TEAD1, in patients younger than 50, were lower than those in patients older than 50, which were totally opposed to the expression of Mst1, Lats1, Oct4 and CDX2. Compared with patients without lymphatic metastasis, the expression of Mst1, Lats1, Oct4 and CDX2 in patients with lymphatic metastasis decreased by 45.91%, 34.78%, 40.91%, 18.92% respectively, and the differences were statistically significant (P<0.05). On the contrary, the expression of YAP1, TAZ and TEAD1 in patients with lymphatic metastasis were 2.47, 2.47, 1.92 times higher than those in patient without lymphatic metastasis, and the differences were statistically significant (P<0.01). As shown in Table 4, expressions of Hippo signaling molecules were significantly associated with gastric cancer TNM stage. In TNM III and IV stage. The mRNA expression of Mst1, Lats1, Oct4 and CDX2 were lower than those in TNM I and II stage (P<0.05). However, compared with TNM I and II stage, the expression of YAP1, TAZ, TEAD1 in TNM III and IV stage increased by 1.48, 1.95, 1.17 times respectively (P<0.01).

The relevance of Hippo signaling molecules expression in GC

From Table 5, we found that in gastric carcinoma the expression of Mst1 and Lats1 were positively correlated (r=0.483, P=0.001), and the expression of Mst1 had a negative correlation with the expression of YAP1(r=-0.507, P=0.000), TEAD1 (r=-0.709, P=0.000) and CDX2 (r=-0.307, P=0.017). The expression of Lats1 had a negatively correlation with the expression of YAP1 (r=-0.637, P=0.000), TEAD1 (r=-0.462, P=0.000) and

Table 5. The Relevance of Hippo Signaling Molecules Expression in GC in Immunohistochemistry

	Mst(+)	Mst1(-)	r	P		Mst1(+)	Mst1(-)	r	P
Lats1(+)	7	4	0.483	0.001	Yap1(+)	6	34	-0.507	0
Lats1(-)	6	43			Yap1(+)	13	7		
	Lats1(+)	Lats1(-)	r	P		Yap1(+)	Yap1(-)	r	P
Yap1(+)	3	29	-0.637	0	CDX2(+)	18	9	0.332	0.019
Yap1(-)	20	8			CDX2(-)	11	22		
	TEAD1(+)	TEAD1(-)	r	P		TEAD1(+)	TEAD1(-)	r	P
CDX2(+)	27	6	0.631	0	Yap1(+)	33	5	0.72	0
CDX2(-)	5	22			Yap1(-)	3	19		
	TEAD1(+)	TEAD1(-)	r	P		TEAD1(+)	TEAD1(-)	r	P
Mst1(+)	6	16	-0.709	0	Lats1(+)	3	7	-0.462	0
Mst1(-)	36	2			Lats1(-)	42	8		
	CDX2(+)	CDX2(-)	r	P		CDX2(+)	CDX2(-)	r	P
Mst1(+)	5	23	-0.307	0.017	Lats1(+)	9	28	-0.609	0
Mst1(-)	15	17			Lats1(-)	20	3		

CDX2 (r=-0.609, *P*=0.000). The expression of YAP1 had a positive correlation with the expression of CDX2 (r=0.332, *P*=0.019) and TEAD1 (r=0.720, *P*=0.000). The expression of TEAD1 was positively correlated with the expression of CDX2 (r=0.631, *P*=0.000).

Discussion

Hippo signaling pathway, a tumor-suppressive pathway, functions to govern tissue growth and restrict organ size by inhibiting cell growth, proliferation and promoting cell apoptosis. Once inactivated, the downstream components in this pathway, such as YAP1, TAZ, will be activated, which lead to tumorigenesis at last. Under normal circumstances, in mammals, the upstream molecules of Hippo signaling pathway, such as Mst1, Last1, Mob and WW45, often form a conserved kinase cassette. These molecules can phosphorylate and inactivate the YAP/TAZ complex on multiple HxRxxS motifs in response to cell density, of which (YAP S127 and TAZ S89) serves as a 14-3-3-binding site and plays the most critical role in regulating nucleus-cytoplasmic translocation (Pan, 2010). When YAP transfers into the nucleus, it will combine with the TEAD family transcription factors to regulate cell growth and apoptosis.

Mst1 belongs to the STE20 (Sterile20) family of protein kinases. The N-terminal region contains a Ser/Thr protein kinase domain while coiled-coil domains are predicted in the central and C-terminal regions. The C-terminal coiled-coil region is referred to as the SARAH (Sav/ Rassf/ Hpo) domain (Zeng et al., 2008). While Lats1 belongs to the NDR (Nuclear Dbf-2-related) protein kinase family with the Ser/Thr protein kinase domain in the C-terminal region (Zeng et al., 2008).In mammals, Mst1 phosphorylates three other core molecules. Lats1 is phosphorylated by Mst1 on the activation loop and hydrophobic motif, possibly with auto-phosphorylation involved (Zhao et al., 2008). In Hippo signaling pathway, Mst1 and Lats1 are important anti-oncogenes. It is reported that Mst1 is required for the maintenance of hepatocyte quiescence in the adult liver, and 70% of human HCC patients show a notably decreased expression of Mst1 (Zheng et al., 2011). In the retina cells with the loss of Mst1, cells endure a excessive proliferation, which can be explained by loss of the ability of Mst1 in inhibiting cell growth and the over expression of CyclinE (Harvey et al., 2003). Related research has suggested that the knockdown of Lats1 in mice can result in ovarian tumor and soft tissue sarcoma (Visser-Grieve et al., 2011). The super-methylation of Lats1 is associated with astrocytoma and mammary cancer (Visser-Grieve et al., 2011). In our experiment, both the mRNA transcription levels and protein levels of Mst1 and Last1 are gradually increased in gastric cancer tissues, adjacent non-tumor tissues, CAG tissues with IM, and normal gastric tissues. Meanwhile, the expression showed a decrease tendency with the increase of lymphatic metastasis and tumor TNM stage.

YAP, the mammalian ortholog of Drosophila Yorkie (Yki), is a candidate oncogene that target genes involved in the regulation of cellular cycle, proliferation and apoptosis. TAZ, a YAP paralog, is identified as a 14-3-3 binding protein. It is said that TAZ, approximately, has 50% sequence identity and similar topology with YAP, though the differences are the lack of N-terminal proline-rich domain, the second WW domain, and the SH3 binding motif (Zhao et al., 2008). This suggests that TAZ may play a similar role with YAP in Hippo signaling pathway. The transcriptional activity of YAP/TAZ is determined by cytoplasm-nucleus shifting. In the cytoplasm, YAP/TAZ is phosphorylated and inhibited by a complex, formed by Mst1, LATS1, Mob, WW45, and the phosphorylation results in their association with 14-3-3 protein and cytoplasmic localization (Zhao et al., 2007). In the nucleus, YAP/TAZ bound to the TEAD family transcription factors and then formed a complex, which bound to the specific DNA promoter and initiate gene transcription (Xu et al., 2011). This cytoplasm-nucleus shifting is involved in cell growth regulation in response to cell-cell contact and cell density. Inactivation of Hippo signaling pathway resulted in nuclear translocation of YAP/TAZ, drove cell proliferation, inhibited cell apoptosis, and eventually led to tumorigenesis (Xu et al., 2011). Some researchers have reported that YAP1 protein increased and localized in nuclear in some human liver and prostate cancers. Also, there are several studies that have demonstrated that overexpression of

YAP1 in the liver led to the dramatic increase of cell proliferation and organ size. Our study shows that the mRNA levels of YAP1, TAZ, TEAD1 in gastric tissues are higher than those in adjacent non-tumor tissues, in CAG tissues with IM, and in normal gastric tissues. And the protein levels of YAP1 and TEAD1 in Western Blot analysis and immunohistochemistry show an increase trend from normal gastric tissues, adjacent non-tumor tissues to GC tissues. All this suggest that YAP1, TAZ, and TEAD1 promoted the development and progression in GC. The expressions of YAP1, TAZ and TEAD1 in GC with lymph node metastasis were significantly higher than those without lymph node metastasis, which suggest that these three factors may play an important role in the metastasis of GC. The expression of YAP1 shows a positive correlation with TAZ and TEAD1 and a negative correlation with Mst1 and Lats1. The expression of TEAD1 shows a negative correlation with Mst1 and Lats1. All this tell us that the core components in Hippo signaling pathway may restrict and promote each other in regulating the development, progression and metastasis of gastric cancer.

CDX2 is a member of the caudal-related homeobox gene family and is intestine-specific transcriptional factor (Kang et al., 2011). In humans, expression of CDX2 is strictly confined to the gut, from the duodenum to the rectum. Though normal gastric mucosa does not express CDX2, aberrant expression of CDX2 is observed in animal and human gastricintestinal metaplasia (Kang et al., 2011). These findings suggest that CDX2 may play the vital role in the development of IM in the human stomach. Relevant researches have reported that CDX2 is highly expressed in human esophageal cells (David et al., 2009) through the interaction with Notch signal pathway and is frequently methylated in lung cancer serving as a Wnt signaling inhibitor (Liu et al., 2012). In our study, we have discovered that the mRNA level of CDX2 in CAG tissues with IM is much higher than that in GC tissues, the latter of which is also higher than that in adjacent non-tumor tissues. However, there was no expression of CDX2 in normal gastric tissues. All these were consistent with the protein expression of CDX2 in Western Blot analysis and Immunohistochemistry. This abnormal phenomenon suggests that aberrant expression of CDX2 may be the initiating event of IM in gastric mucosa. Related researchers have reported that the specific expression of CDX2 gene in intestinal type GC may be a dynamic process: no expression in normal gastric tissues → abnormal high level in gastric tissue with IM → no expression in the process of tumorigenesis → the second high level in intestinal type GC (Chu et al., 2011). In our study, we also found the similar phenomenon. We discovered that the expression of CDX2 had a negative correlation with lymphatic metastasis and tumor TNM Stage, which suggested that the aberrant expression of CDX2 could inhibit the metastasis of GC. Therefore, we can further conclude that in GC the role of CDX2 is similar to anti-oncogene, to some extent.

The Oct3/4 gene, a POU (Pit-Oct-Unc) family of transcription factors is thought to be expressed only in embryonic stem cells and in tumor cells (Trosko, 2006) and associated with the proliferation and self-renew of cells. Junko Matsuoka (Matsuoka et al., 2012) had told us that Oct3/4 expression is implicated in self-renewal and tumorigenesis via activation of its downstream genes in cancer-stem like cells. There are other studies that have reported that Oct4 is closely related to early stage of pancreatic cancer carcinogenesis (Wen et al., 2012). In our research, we discovered that the expression of Oct4 presented an increase trend from the GC tissues, adjacent non-tumor tissues, CAG tissues with IM to normal gastric tissues, and had a negative correlation with lymphatic metastasis and tumor TNM Stage. These findings suggest that Oct4 may suppress the activation of cancer cells. In Noriyuki Nishioka,s research (Nishioka et al., 2009), they have proposed that CDX2 is the major target of TEAD4, and Tead4 is dispensable for CDX2 expression when Oct3/4 levels are reduced, suggesting that Tead4 can induce cdx2 expression by overcoming Oct4 mediated suppression. Our study discovered that the expression of CDX2 had a positive correlation with TEAD1, and a negative correlation with OCT4, which were consistent with the results of Noriyuki Nishioka's research. That is to say Hippo signaling pathway can interact with CDX2 and Oct4 to regulate cell proliferation and apoptosis.

From our experiment, we conclude that in Hippo signaling pathway, Mst1 and Lats1 are tumor suppressor genes, which can suppress the development and metastasis of tumor. The YAP1, TAZ and TEAD1 are oncogenes, which are indispensable in regulating the growth and differentiation of normal organism, but also can initiate the formation of carcinoma in the specified conditions. The gene CDX2 can initiate intestinal metaplasia in gastric mucosa, which is the precancerous lesion for GC. And in gastric, the gene CDX2 is the main target of Hippo signaling pathway in nucleus. The gene Oct4 can suppress the tumorigenicity of cancer cells and play a vital role in regulating the expression of CDX2 in Hippo signaling pathway.

All summarized, Hippo signaling pathway plays a crucial role in the development, progression and metastasis of gastric cancer, and manipulation of the main components expression in this pathway may be a potential therapeutic strategy for gastric cancer. We believe that in a few years, one can expect exciting discoveries in Hippo signaling pathway that provide new targets for treatment of gastric cancer.

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