

Supplemental Data

Homeobox D10 Gene, a Candidate Tumor Suppressor, Is Downregulated through Promoter Hypermethylation and Associated with Gastric Carcinogenesis

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Supplementary Table 1. Primer sequences used for quantitative real-time PCR

Gene	Primer	Sequence	PCR product (bp)
<i>HCLS1</i>	Forward	5' GCCCTTGCTGCCCATAG 3'	119
	Reverse	5' CAGGCTCTGCTTCGTACACT 3'	
<i>ANP32A</i>	Forward	5' CCTCCCGCAACTCACATA 3'	297
	Reverse	5' CGCTTCTGACCCCTTCT 3'	
<i>PDGFRL</i>	Forward	5' GTCTGGCTGCTGCTTGGT 3'	189
	Reverse	5' CTGCGTCTTTGGTGCTGA 3'	
<i>IGFBP3</i>	Forward	5' GGCCATGACTGAGGAAAGGA 3'	85
	Reverse	5' CCTGACTTTGCCAGACCTTCTT 3'	
<i>CXCL9</i>	Forward	5' GGCATCATCTTGCTGGTT 3'	97
	Reverse	5' GGTGGATAGTCCCTTGGT 3'	
<i>RAC2</i>	Forward	5' GATGGTGGACAGCAAGCC 3'	121
	Reverse	5' GAGGGAGAAGCAGATGAGGAAG 3'	
<i>KRT5</i>	Forward	5' CGATGACCTCCGCAACAC 3'	340
	Reverse	5' GACTGGTCCAACCTCTCTC 3'	
<i>NTS</i>	Forward	5' AAGCACATGTTCCCTCTT 3'	318
	Reverse	5' CATAAGCTGCCGTTTCAGA 3'	
<i>PAGE4</i>	Forward	5' TCTCCCTTCATTCCTCGC 3'	182
	Reverse	5' AGTTGGTGGTTCCTCTTGC 3'	
<i>KRT18</i>	Forward	5' AAGAACCACGAAGAGGAAGTA 3'	363
	Reverse	5' CATCTGTAGGGCGTAGCG 3'	
<i>HoxD10</i>	Forward	5' AAAGTCTCCCAGGTGGAGAG 3'	142
	Reverse	5' TGCTGGTTGGTGTATCAGAC 3'	
<i>GAPDH</i>	Forward	5' GGCTCAGTTTGTGGCAGAATC 3'	101
	Reverse	5' CATCGCCTTCGAAGTTGCT 3'	

HOXD10 IS DOWNREGULATED IN GASTRIC CANCER

Supplementary Table 2. Primers of IGFBP3 used for quantitative real-time PCR in ChIP analysis

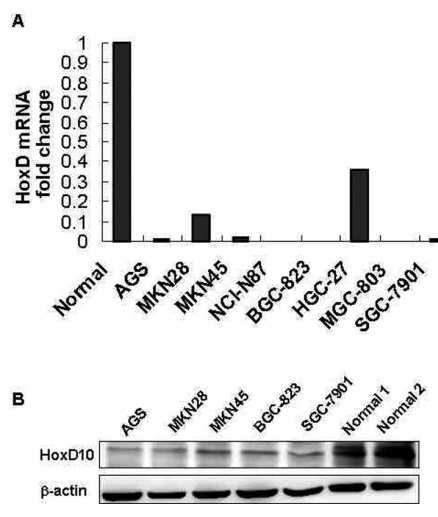
ChIP name	Predicted bind sequence	primer (5'-3')	PCR size
A1	AATAAAACAA (H1)	(F) CATTGGGCACTGAACAAG	179
	AATAAAAAGT (H2)	(R) AGCCTGGACTGACCACT	
A2	TCITTTTATT (H3)	(F) CATAAGAAAATGACGGTGCT	133
		(R) TGGAAAAGATCAATTCGTCC	
A3	ATTGCTATT (H4)	(F) AAGATTAACCTCACCCAAGGC	107
		(R) AGGTGGATAGGTGACTTG	
A4	CTTTATTATT (H5)	(F) GCCGACAGGAGTTACAG	277
		(R) TGTTCTTCTGTCTGGGTA	

Supplementary Table 3. Clinico-pathologic features of *HoxD10* in gastric cancer and normal stomach

Characteristics	Normal group	Gastric cancer	p value
Gender n (%)			0.500
Male	18 (48.9)	19 (44.4)	
Female	15 (51.1)	14 (55.6)	
Age (mean ± SD)	48.27 ± 10.71	53.64 ± 10.80	0.497
TNM stage n (%)			
I		4 (12.1)	
II		6 (18.2)	
III		7 (21.2)	
IV		16 (48.5)	
Differentiation			
Poor (or no differentiation)		22 (66.7)	
Well or moderate		11 (33.3)	

Supplementary Table 4. Fold change (Log₂) of selected genes in *HoxD10* transfectants detected by cDNA microarray and qRT-PCR (*HoxD10* versus Vector control)

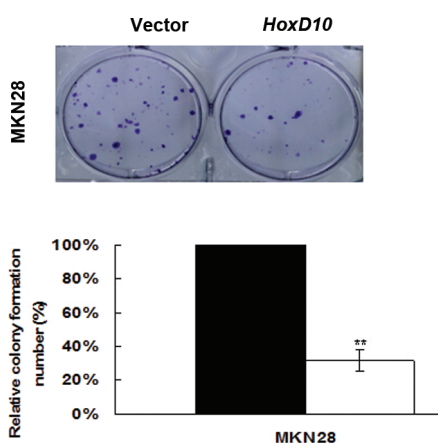
Gene Symbol	Gene description	cDNA microarray Fold change	qRT-PCR Fold change
<i>HCLS1</i>	hematopoietic cell-specific Lyn substrate 1	2.3493805	2.927825
<i>ANP32A</i>	acidic (leucine-rich) nuclear phosphoprotein 32 family, member A	1.5278893	0.785913
<i>PDGFRL</i>	platelet-derived growth factor receptor-like	1.4941043	1.724332
<i>IGFBP3</i>	insulin-like growth factor binding protein 3	1.2196761	2.531675
<i>CXCL9</i>	chemokine (C-X-C motif) ligand 9	1.1326157	3.626737
<i>RAC2</i>	ras-related C3 botulinum toxin substrate 2	-2.6205406	-1.28035
<i>KRT5</i>	keratin 5	-1.9828073	-2.31552
<i>NTS</i>	neurotensin	-1.98145	-0.51863
<i>TUSC3</i>	tumor suppressor candidate 3	-1.290414	-0.68204



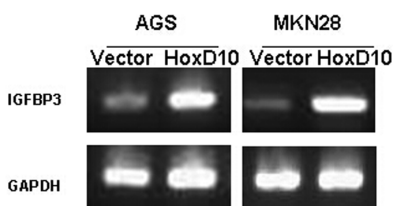
Supplementary Figure 1. (A) The relative expression of *HoxD10* mRNA in eight gastric cancer cell lines (AGS, MKN28, MKN45, NCI-N87, BGC823, HGC27, MGC803, and SGC7901) was analyzed by quantitative real-time RT-PCR. The normal stomach tissue is normalized as 1. (B) The expression of *HoxD10* protein in gastric cancer cells were compared to normal stomach tissues. Normal: normal stomach tissue.

Supplementary Table 5. Clinico-pathologic features of *HoxD10* methylation in gastric cancer

Characteristics	Methylated n (%)	Unmethylated n (%)	p value
Surgical samples			
Gender			0.802
Male	24 (48.9)	4 (44.4)	
Female	25 (51.1)	5 (55.6)	
Lauren type			0.631
Intestinal	17 (51.5)	2 (40.0)	
Diffused	16 (48.5)	3 (60.0)	
TNM stage			0.031
I	6 (12.7)	5 (55.6)	
II	8 (17.0)	1 (11.1)	
III	18 (38.3)	2 (22.2)	
IV	15 (31.0)	1 (11.1)	
Differentiation			0.042
Poor (or no differentiation)	15 (56.7)	6 (100)	
Well or moderate	6 (43.3)	0 (0)	
Biopsy samples			
Gender			0.583
Male	14 (58.3)	2 (50.0)	
Female	10 (41.7)	2 (50.0)	
TNM stage			0.534
I	0 (0)	0 (0)	
II	5 (20.8)	0 (0)	
III	7 (29.2)	1 (25.0)	
IV	12 (50.0)	3 (75.0)	
Differentiation			0.613
Poor (or no differentiation)	16 (66.7)	3 (75.0)	
Well or moderate	8 (33.3)	1 (25.0)	



Supplementary Figure 2. The surviving monolayer colonies were stained in MKN28 cells after selected with G418. The numbers of colonies in *HoxD10*-transfected cells were presented as mean percentage \pm SD in low bar diagram, pcDNA3.1 vector transfectants normalized as 100%. ■, Vector; □, *HoxD10*.



Supplementary Figure 3. The expression of IGFBP3 mRNA was detected after transfection with *HoxD10* or control vector in gastric cancer cell lines (AGS and MKN28) by conventional RT-PCR. GAPDH was used as the internal control.