

**HMGA2 promotes vasculogenic mimicry and tumor aggressiveness
by upregulating Twist1 in gastric carcinoma**

Junying Sun^{1,#}, Baocun Sun^{1,2,3,#*}, Ran Sun⁴, Dongwang Zhu⁵, Xiulan Zhao^{1,2}, Yanhui Zhang³, Xueyi Dong¹, Na Che^{1,2}, Jing Li^{1,2}, Fang Liu¹, Nan Zhao^{1,2}, Yong Wang³, Danfang Zhang^{1,2}

¹ Department of Pathology, Tianjin Medical University, Tianjin 300070, China

² Department of Pathology, Tianjin General Hospital, Tianjin Medical University, Tianjin 300052, China

³ Department of Pathology, Tianjin Cancer Hospital, Tianjin Medical University, Tianjin 300060, China

⁴ Department of Surgery, Tianjin Nankai Hospital, Tianjin 300100, China

⁵ Department of Prosthodontics, Affiliated Stomatological Hospital, Tianjin Medical University, Tianjin 300070, China

#These authors equally contribute to this study.

***Corresponding author:**

Prof. Baocun Sun,

Department of Pathology and General Hospital and Cancer Hospital of Tianjin Medical University, Tianjin 300070, PR China;

E-mail: baocunsun@aliyun.com

Tel: 86-13602111192

Fax: 86-22-83336813

Mailing Address : No.22 Qixiangtai Road, Heping District, Tianjin
300070, China

Table S1. Antibodies used in this study

Antibody	Source	IHC Concentration/ WB	Product Number	Manufacture
HMGA2	Rabbit	1:100 (IHC) 1:500(WB)	Ab-52039	Abcam
Twist1	Rabbit	1:200 (IHC) 1:200(WB)	Sc-15393	Santa Cruz
VE-cadherin	Rabbit	1:500(WB) 1:400(IHC)	ab33168	Abcam
E-cadherin	Mouse	1:200(WB) 1:100(IHC)	ab1416	Abcam
N-cadherin	Rabbit	1:1000(WB)	AF4039	Affinity Biosciences
Vimentin	Rabbit	1:500(WB) 1:200(IHC)	ab92547	Abcam
MMP2	Rabbit	1:200(WB) 1:100(IHC)	10373-2-AP	LuoSai-BIO
MMP9	Rabbit	1:500(WB)	ab76003	Abcam
GAPDH	Rabbit	1:2000(WB)	Sc-25778	Santa Cruz
CD34	Mouse	1:550(IHC)	Zm-0046	ZSGB-Bio
Endomucin	Rat	1:800(IHC)	14-5851-81	Ebioscience

Note: IHC: Immunohistochemistry, WB: Western blot.

Table S2. The sequences of primer used for Semi-quantitative RT-PCR

Gene	Forward primer(5'-3')	Reverse primer(5'-3')
Twist1	CCATCCACACCGTCCCCTCCCCCTC	TCTGGCTCTTCCTCGCTGTTGCT
CDH5	ACAAGGACATAACACCACGAAACG	TGAGATGACCACGGGTAGGAAGT
HMGA2	CCCAAAGGCAGCAAAAACA A	GCCTCTTGGCCGTTTTTCTC
GAPDH	CCTGGCCAAGGTCATCCATGAC	TGTCATACCAGGAAATGAGCTTG

Table S3. The sequences of primer used for ChIP-q PCR

Primer name	Forward primer (5'-3')	Reverse primer (5'-3')
Primer1	CCCACTGTGTAGAAGCTGTTG	GAAGCGGTCGGAGGAGA
Primer2	GTCACAATGCGGAGCCTAAT	AAACCCAGTCCATGGGAAAG
Primer3	GGTTAATGGGCAGCTAAGACA	CCTGTAGAAAGTGCAGGATCAG
Primer4	CTCATGATGTCAGGCGGTTT	ACGGAGTTCTGTTTGGGTTTC
Primer5	GCGGTGCCAAGTGTCAG	GTCTCCGGAAATTCGAGGTTG
Primer6	GAAGGTGTGTAATTGGAGTTAAGG	CTCCTCTGTGCCAGTGATT

Fig.S1 The expression of HMGA2 in GC cell lines and its relation of Twist1, VE-cadherin in GC patients.

(A)Western blot showed the expression of HMGA2 expression in various GC cell lines (*p < 0.05) (B) Immunofluorescence staining showed the expression of HMGA2 in cells which formed VM on matrigel.(C)The relation of HGMA2 and Twist1, VE-cadherin in GC patients and they are significantly related to poor prognosis (*P<0.05)

Fig.S1

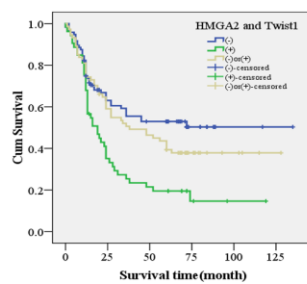
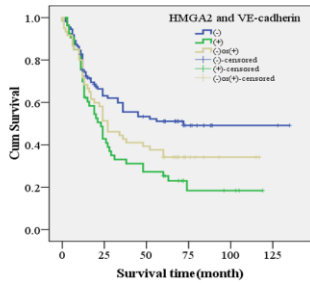
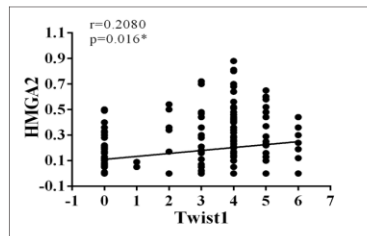
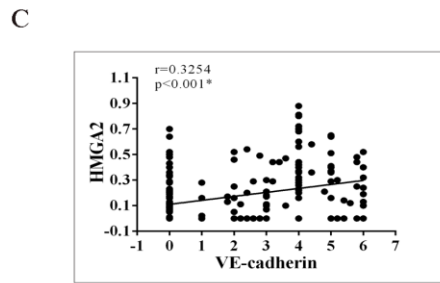
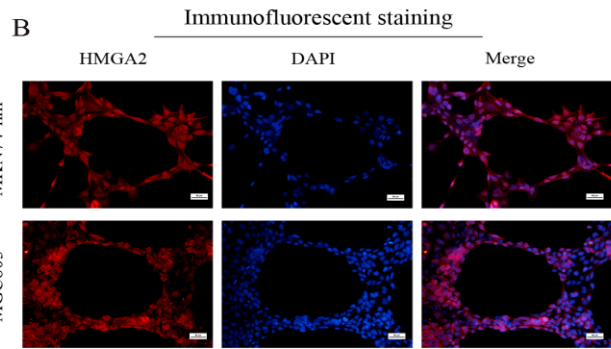
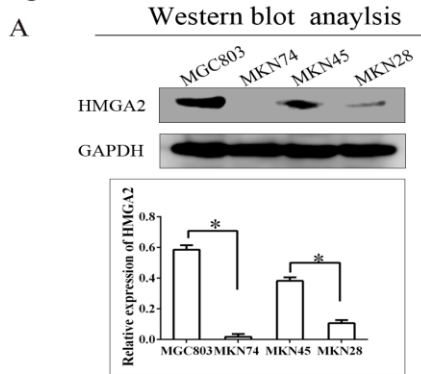


Fig.S2

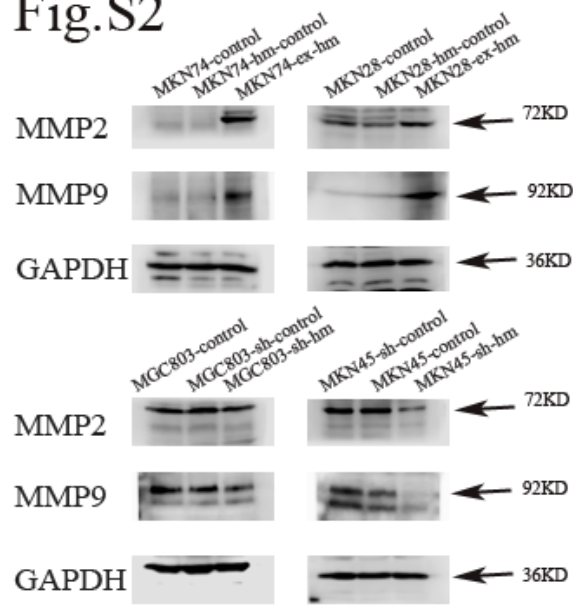


Fig.S3

