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Effects of hepatocyte growth factor/scatter factor on the invasion of colorectal cancer cells *in vitro*

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Abstract

AIM: Hepatocyte growth factor (HGF) is a multifunctional growth factor which has pleiotrophic biological effects on epithelial cells, such as proliferation, motogenesis, invasiveness and morphogenesis. There are few reports about the role of HGF played in the colorectal cancer invasion. In the present study, we tried to investigate the possible mechanism of HGF involved in the invasion of colorectal cancer cells *in vitro*.

METHODS: Matrigel migration assay was used to analyze the migrational ability of Caco-2 and Colo320 *in vitro*. We detected the mRNA expressive levels of MMP-2, MMP-9 and their natural inhibitors TIMP-1, TIMP-2 in Caco-2 cells by reverse-transcription polymerase chain reaction (PCR) technique.

RESULTS: After 48 h incubation, there were notable differences when we compared the migrational numbers of Caco-2 cells in the group of HGF and PD98059 (the inhibitor of p42/p44MAPK) with the control (104.40±4.77 *vs* 126.80±5.40, $t = 7.17$, $P = 0.002<0.01$; 104.40±4.77 *vs* 82.80±4.15, $t = 7.96$, $P = 0.001<0.01$). The deviation between the HGF and PD98059 was significant ($P<0.01$). Compared with controls, MMP-2 and MMP-9 mRNA expres-sions were up-regulated by HGF (0.997±0.011 *vs* 1.207±0.003, $t = 35.002$, $P = 0.001<0.01$; 0.387±0.128 *vs* 0.971±0.147, $t = 106.036$, $P = 0.0000<0.01$, respectively); compared with controls, TIMP-1, TIMP-2 mRNA expressions were increased by PD98059 (1.344±0.007 *vs* 1.905±0.049, $t = 17.541$, $P = 0.003<0.01$; 1.286±0.020 *vs* 1.887±0.022, $t = 24.623$, $P = 0.002<0.01$, respectively).

CONCLUSION: HGF promoted Caco-2 migration mainly by p42/p44MAPK pathway; HGF/SF stimulated the expression of MMP-2, MMP-9 in Caco-2 and enabled tumoral cells to damage the ECM and reach the distant organ and develop metastasis; HGF played the function of promoted-invasion and promoted-metastasis, in which cellular selection was possible.

Key words: HGF; Colorectal cancer cells; Invasion; MMPs

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INTRODUCTION

Hepatocyte growth factor/scatter factor is a polypeptide growth factor, which enhances strong cellular disintegration, tissue formation, inducing the migration, invasion and angiogenesis of epithelial cells[1]. The protein production of the c-met proto-oncogene encodes trans-membrane tyrosine kinase and is the receptor for hepatocyte growth factor, which regulates proliferation, differentiation, morphogenesis and motility in various cells after being activated by HGF. Thus, it relates with the genesis and progress in many types of human tumors. The mechanism for HGF-c-met in the invasion and metastasis of malignant tumor includes that they can promote cellular migration and increase the abilities of invasion, they also can trigger Ca^{++}-dependent signaling system, so activate the Ras, then activate extracellular-signal regulated kinase (Erk), thereby regulate the contraction and motion of cells and phosphorylate microfilament relating-protein, regulate cellular skeleton and reinforce the ability of cellular movement. HGF can coordinate other factors *in vivo* and therefore enlighten the abilities of migration and invasion in some malignant cells[2]. Yi found that HGF could increase the invasion of 13 lung cancer cells in which the receptor of c-met was expressed[3]. The motility of tumoral cells correlated closely with the metastasis in tumor.

Tumoral invasion occurs in three main steps: adhesion, degradation of basement membrane and movement, in which the degradation of basement membrane passes mainly through proteolytic kinases. Matrix metalloproteinases (MMPs) are part of these kinases, in which MMP-2 and MMP-9 correlated with the tumoral metastasis. The report showed that HGF could regulate the expression of MMPs.

Mitogen-activated protein kinases (MAPK) are the kinases between the receptors of cell membrane and the significant intracellular regulating-targets. Cells apply this system conducting extracellular stimulating-signal to the nucleus of cell so that the biological effects from the cells can be trasmitted. The pathway of intracellular signal conduction for HGF-c-met induced-biological effects is not clear.

Cellular migration is the premise of tumoral metastasis. The characters of tumoral infiltration and metastasis are the key factors affecting the survival and prognosis in patients. To

confirm the conclusion that HGF can promote migration *in vitro*, regulate the expression of MMPs and exist the MAPK pathway during these processes, we applied the HGF together with the inhibitors of p42/p44MAPK(PD98059) and p38MAPK(SB203580) dealt with the colorectal cancer cells: in Caco-2 and Colo320, we also observed mRNA expression of MMP-2, MMP-9 and tissue inhibitor of metalloproteinase-1 (TIMP-1) and tissue inhibitor of metalloproteinase-2(TIMP-2) in order to investigate the function of HGF in the invasion of colorectal cancer cells *in vitro*.

MATERIALS AND METHODS

Materials

Cells and cell culture The human colorectal cancer cell lines Caco-2 and Colo320 were purchased from China Academy of Science in Shanghai Institute of Life Science, Caco-2 were cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/mL penicillin and 100 μg/mL streptomycin at 37 ℃ in a 50 mL/L CO_2 atmosphere. Medium were exchanged every other day, when the cells reached confluence, we digested them with 0.02% EDTA+trypsin and delivered them according to 1∶2 or 1:4∶. Colo320 were cultured in DMEM supplemented with 20% fetal bovine serum, others were identical with Caco-2.

Reagent and apparatus DMEM were purchased from Gibcobrl; FBS from TBD; HGF from JingMei Biol in ShenZhen and were compounded with free serum medium, the concentration was 20 ng/mL. PD98059 and SB203580 were from Promega, they were compounded with DMSO and made up to 10 and 20 mmol/L for storage and the applicable concentration was 40 μmmol/L combined with free serum, stored at 4 ℃. Matrigel from Gene Company in ShenYang was compounded with DMEM according to 1∶3, 0 ℃ fusion; 8 μmmol/L polycarbonate size membrane and TRIzol were from HuaMei Company, Boyden chamber were from the Department of Oncology in China Medical University. RT-PCR kit was from Takara, the primers of MMP-2, MMP-9, TIMP-1, TIMP-2, β-actin were synthesized by BoYa Bio Company in Shanghai. Amplified sections were 307, 215, 285, 265, and 690 bp respectively. Autoradiography were purchased from Olympus and PCR amplification instrument were from PE Company in the USA.

Methods

Matrigel migration assay This assay is similar to that described previously[5], Boyden chambers system whose upper and lower compartments were used to analyze the invasive ability of Caco-2 and Colo320. The chambers were washed, dried and irradiated under ultraviolet rays for 30 min, the experiments were divided into four groups DMEM, HGF, PD98059, and SB203580.

DMEM, 20 ng/mL HGF, 10% FBS (Caco-2) or 20% FBS (Colo320), 200 μL in the lower compartment with 50 μg Matrigel onto a polycarbonate membrane, and then 1 mL (about 3 ± 10^5 cell/mL) colorectal cancer cells were put in the upper compartment of the chamber, and the chambers were incubated at 37 ℃ in humidified air containing 50 mL/L CO_2 for 48 h, at the end of incubation, the cells on the upper side of polycarbonate membrane were wiped off with a cotton swab and the remaining cells that traversed the Matrigel and spread on the lower surface of the membrane were rinsed using distilled water twice, the membrane were fixed for 30 min with 5 mL methanol, dried and stained with hematoxylin for 10 min, rinsed with 1% HCl+10% alcohol and distilled water for 1-5 min, rinsed with 1% ammonia water and distilled water for 1-5 min, stained with eosin 1-10 min, at last rinsed and fixed with gradient alcohol and xylene, enclosed and counted five fields of vision using light microscope. Results were expressed as the number of colorectal cancer cells invaded per filter. Experiments were done in triplicate and results are shown as mean±SD.

Effect of PD98059 on HGF-induced migration 40 μmol/L PD98059 were added to the upper compartment with 1 mL cells for 30 min before 20 ng/mL HGF was added to the lower side of chamber which was incubated at 37 ℃ in a 50 mL/L CO_2 atmosphere for 48 h. After incubation, similar treatment as the above-mentioned, was carried out.

Effect of SB203580 on HGF-induced migration: 40 μmol/L SB203580 were added to the upper compartment with 1 mL cells for 30 min before 20 ng/mL HGF was added to the lower side of chamber which was incubated at 37 ℃ in a 50 mL/L CO_2 atmosphere for 48 h. After incubation, the treatment was identical with the above-mentioned.

Detection of MMP-2, MMP-9, TIMP-1, TIMP-2 mRNA by RT-PCR

Total RNA was extracted from subconfluent cell layers according to Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction, 2 μL RNA was reverse-transcribed using AMV, 3 μL of the reaction were used for amplification. The primers used are as follows:

MMP-2-F: 5'-TCAACGGTCGGGAATACA-3'
MMP-2-R: 5'-CCCACAGTGGACATAGCG-3'
MMP-9-F: 5'-TCGAACTTTGACAGCGACAAGAA-3'
MMP-9-R: 5'-TCAGGGCGAGGACCATAGAGG-3'
TIMP-1-F: 5'-CTTCCACAGGTCCCACAACC-3'
TIMP-1-R: 5'-CAGCCCTGGCTCCCGAGGC-3'
TIMP-2-F: 5'-AAACGACATTTATGGCAACCCT-ATC-3'
TIMP-2-R: 5'-ACAGGAGCCGTCACTTCTCTTG-ATG-3'
β-actin-R: 5'-GATTGCCTCAGGACATTTCTG-3'
β-actin-F: 5'-GATTGCTCAGGACATTTCTG-3

PCR reaction conditions were as follows: Thirty-five cycles of MMP-2 PCR were performed, each consisting of denaturation for 3 min and 45 s at 94 ℃, annealing for 1 min at 55 ℃ and elongation for 1 and 7 min at 72 ℃. Thirty-five cycles of MMP-9 PCR were performed, each consisting of denaturation for 3 min and 45 s at 94 ℃, annealing for 1 min at 60.5 ℃ and elongation for 1 and 7 min at 72 ℃. Thirty cycles of TIMP-1 PCR were performed, each consisting of denaturation for 3 min and 30 s at 94 ℃, annealing for 1 min at 63 ℃ and elongation for 30 s and 7 min at 72 ℃. Thirty cycles of TIMP-2 PCR were performed, each consisting of denaturation for 3 min and 45 s at 94 ℃, annealing for 1 min at 65 ℃ and elongation for 30 s and 7 min at 72 ℃. Agarose gel electrophoresis consisting of 20 g/L ethidium bromide (EB) was used to analyze the production of PCR. Electrophoresis zones were reserved for 1 D kodak autoradiography system, β-actin was for controls; measure values were counted/β-actin.

Statistical analysis

SPSS12.0 software was used, all values were expressed as mean±SD, *t*-test was used to determine the significance of differences in multiple comparisons. Values of $P<0.05$ were considered to be statistically significant.

RESULTS

Migration of Caco-2

After 48 h incubation, there were notable differences when we compared the migrational numbers of Caco-2 cells in the group of HGF and PD98059 with the control ($P<0.01$). The deviation between the HGF and PD98059 was significant ($P<0.01$). The group of SB203580 had no implication. We did not find the phenomenon in the Colo320 cells. HGF increased the number of invading Matrigel cells, the more numbers, the more the invading ability. We found that PD98059 inhibited the migration of HGF-induced Caco-2. (Figure 1 and Table 1).

Table 1 Migrational numbers of Caco-2 in different factors

	The migrational numbers of Caco-2 (mean±SD)
Control	104.40±4.77
HGF/SF	126.80±5.40[b]
PD98059	82.80±4.15[d]
SB203580	108.4±14.38

[b]$P<0.01$, $t = 7.17$; [d]$P<0.01$, $t = 7.96$.

Expression of MMP-2, MMP-9, TIMP-1, TIMP-2 mRNA in Caco-2 cell conditions were identified with Matrigel migration assay .Examination of MMP-2, MMP-9, TIMP-1,TIMP-2 expression were by RT-PCR. DMEM was control, MMP-9 expressed all groups except control, MMP-2 was high expression in HGF group, TIMP-1 and TIMP-2 were high expression in the group of PD98059. HGF could stimulate the expression of MMP-2 and MMP-9, but the function was inhibited in the presence of PD98059 ($P<0.01$).There were different expressions of MMP-2, MMP-9 and TIMP-1, TIMP-2 in the group of PD98059, with no significance. Expression of MMP-2, MMP-9 and TIMP-1, TIMP-2 in the group of HGF were not divergent. (Figure 2 and Table 2).

Table 2 Expression of MMPs and its inhibitors in Caco-2 (mean±SD)

	MMP-2	MMP-9	TIMP-1	TIMP-2
Control	0.997±0.011	0.387±0.128	1.344±0.007	1.286±0.020
HGF	1.207±0.003	0.971±0.147	0.846±0.075	0.992±0.009
PD98059	0.824±0.037	0.350±0.007	1.905±0.049	1.887±0.022

DISCUSSION

In vitro, HGF/SF has the function of strongly promoting mitogen and stimulating the growth of normal and malignant cells[6]. HGF/SF could agitate directly cellular mobility and spreadable effects and colon formation gets diffused by it.

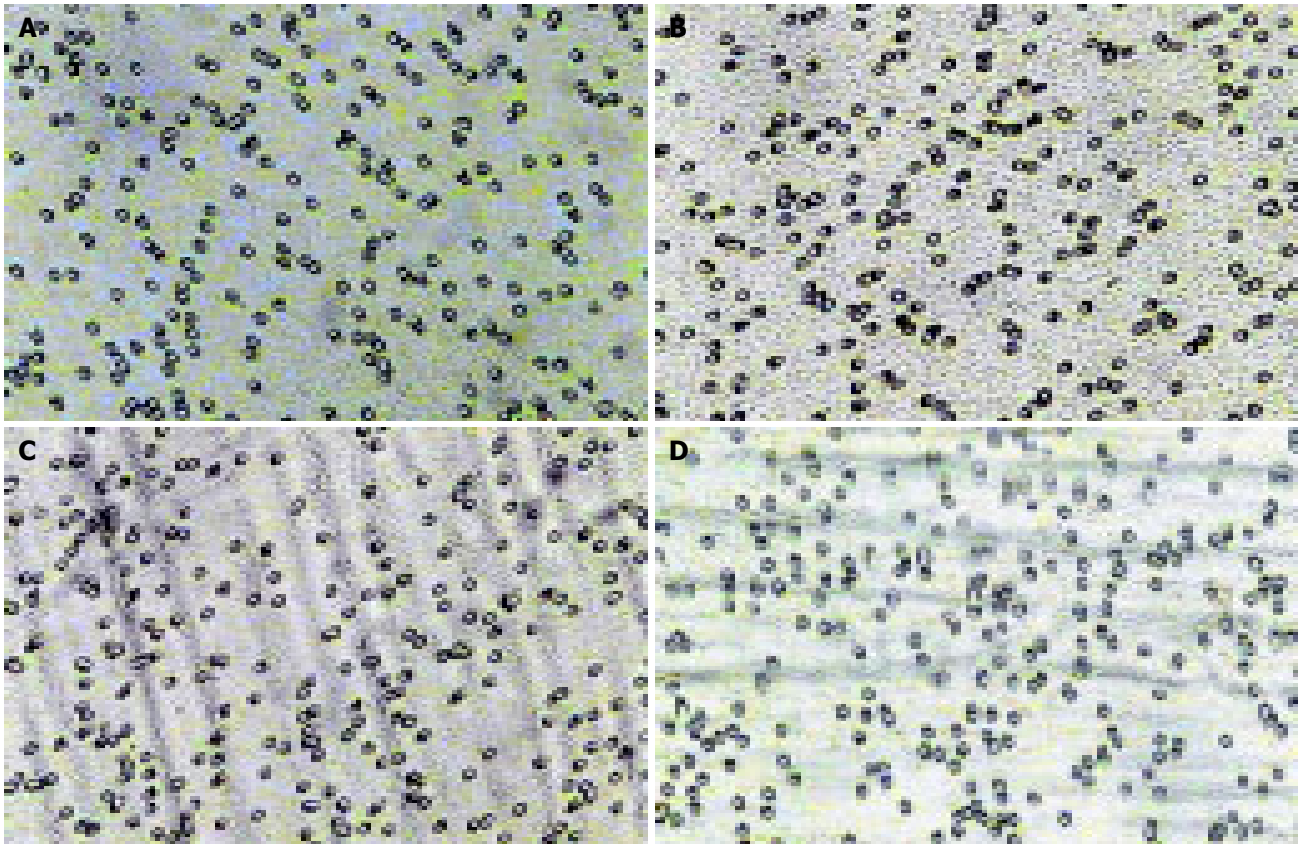

Figure 1 Migration of Caco-2 in different factors: control (**A**), Effect of HGF (**B**), PD98059 (**C**), and SB203580 (**D**).

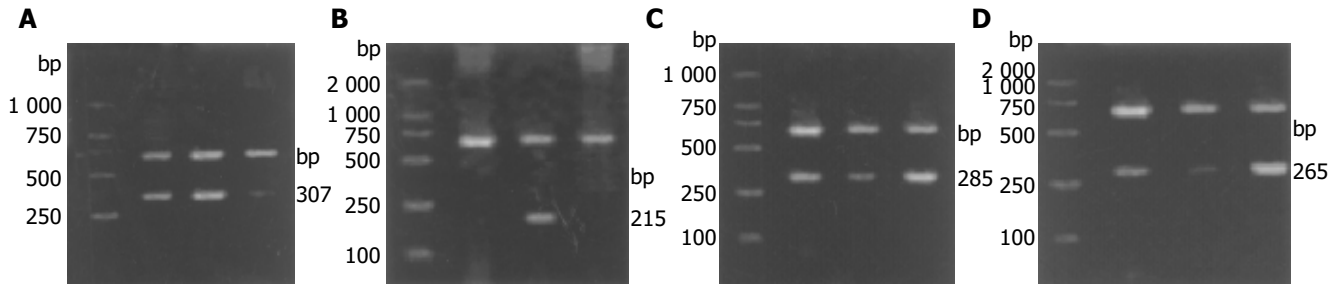

Figure 2 Expression of MMPs and TIMPs in Caco-2 cell: MMP-2 amplification section (**A**), MMP-9 amplification section (**B**), TIMP-1 amplification section (**C**), TIMP-2 amplification section (**D**).

The activity of HGF-promoted motogen and morphogen could promote adhesion, migration and infiltration of the cancer cells[7], as it enhanced Integrins-modulated adhesion so that lymphocyte invasion increased six-fold[8]. About the mechanism of HGF-promoted tumoral metastasis, Fujisaki[9] reported that the stimulation of CD44 could induce the expression of c-met; HGF/SF amplified CD44-induced adhesion of LFA-1 (lymphocyte function associated antigen-1), further amplified and agitated the Integrin-modulated adhesion, enabled the cancer cells to adhere blood vessel endothelium and pass through the vessel walls. The experiments of cotransfection for c-met and CD44 found that CD44, acted as the co-receptor of HGF, promoted the c-met tyrosine kinases phosphorylation and downstream signaling conduction protein kinases activation (activated MAPK), induced tumoral growth and metastasis[10].

Recently, the research of extracellular-signal transduction has made great progress in the interior and exterior parts of the country. Extracellular stimulation entered from the cellular surface and activated the signaling transduction pathway in the cytoplasm, transduced the signaling to the nucleus through many pathways and promoted or inhibited special expression of the target-gene[11], including the pathway of Ras-MAPK which played a main role in promoting cellular proliferation and regulating genetic transcription. The character of the cytokine receptor decided the manner of signaling transduction, many cytokines transmitted to the nucleus through mainly MAPK-cascades, induced the same genetic expression procedures, produced the same biological effects so that MAPK-cascades originated together or from the last pathway in which many membrane receptors transducted growth signaling to pass through the membrane[12]. MAPK was the important intercellular signaling system, cells applied it to transmit stimulating extracellular signal to the nucleus and modulated biological effects produced by cells[13]. It had decided four MAPK pathways in eukaryotic cell, including ERK, JNK, p38MAPK, and ERK5[14]. MAPK was a kind of serine/threonine protein kinase, activated by phosphorylation[15]. An extracellular stimulator could activate synchronously several members of MAPK families, Xia[16]found if they removed neurogenic growth factor from incubation systems, and then induced the apoptosis of PCI2, there existed activation of p38MAPK, JNK and inhibition of ERK, the deduced apoptosis and survival were decided by the balance between ERK-activated by growth factor and p38MAPK, and JNK-activated by stress.

In our study, we found that PD98059, the inhibitor of p42/p44MAPK, could restrain the numbers of HGF-induced colorectal cancer cells passed Matrigel, as such, we confirmed that p42/p44MAPK was one of the pathways of HGF-promoted Caco-2 invasion.

The main components of extracellular matrix (ECM) including collagen, glucoprotein, proteoglycan and glycosamine existed as the form of basement membrane and intercellular tissues. There was a kind of anti-adhesion protein in extracellular matrix called basement membrane protein, the sections of these molecules degradation had chemotaxis so that promoted the mobility of the cancer cells[13]. A lot of barriers were during the metastatic processes, it was of significance for the basement membrane beside tumoral cells and interstitial matrix to maintain tissue morphogenesis and biological specificity. Tumor cells might produce proteolytic ferment through autosecretion or stimulate host to cross basement membrane so that metastasis occurred. Therefore, the degradation of tumor to ECM was the prerequisite for tumoral invasion and metastasis[14]. Matrix metalloproteinases (MMPs) were this kind of peptidase which Zn^{2+}-dependently degraded ECM, at present, 20 members of MMPs families were determined. According to the different substrates, MMPs were divided as follows: interstitial collagenase, gelatinase, stromelysin, membrane-type MMPs[17]. In the 1980s, it was first reported that MMPs related with the potency of cancer metastasis[18]. The mechanism of tumor cells degraded ECM including the activation of MAPK, cells applied the pathway to promote the secretion of MT-MMPs and the rearrangement of microfilament skeleton formed by myoprotein and increased the ability of decomposing collage of ECM and migration, then, induced tumoral infiltration and metastasis. MMPs were the major kinases during the physiological and pathological processes in the rebuilding or degradation of ECM, they were the key to removing the barrier of cellular migration. Increasing activities of MMPs was one of the essential conditions in tumor invasion and metastasis, MMPs could affect cellular adhesion and migration directly. But the total degradation of ECM was controlled strictly by the balance between activated MMPs and TIMPs (inhibitors of MMPs)[19]. Our results showed that HGF/SF up-regulated the expression of MMP-2, MMP-9 and TIMPs in Caco-2 compared with the control groups, we confirmed *in vitro* that HGF increased the numbers of migrational Caco-2, the results suggested that increasing activities of MMPs in the presence of HGF enabled tumoral cells to damage the

ECM and easily developed metastasis.

In conclusion, HGF promoted Caco-2 migration mainly by p42/p44MAPK pathway; HGF/SF up-regulated the expression of MMP-2, MMP-9 in Caco-2 and enabled tumoral cells to damage the ECM; HGF played the function of promoted-invasion and promoted-metastasis in cellular selection; HGF could play the biological effects by MAPK, therefore, it had a major significance for we studied the regulation about MAPK in tumoral metastasis to understand the relationship between the MAPK pathway and colorectal cancer, furthermore, it would provide the evidence for MAPK to become the target of tumoral treatment.

REFERENCES

1 **Zeng Q,** Chen S, You Z, Yang F, Carey TE, Saims D, Wang CY. Hepatocyte growth factor inhits anoikis in head and neck squamous cell carcinoma cells by activation of ERK and Akt signaling independent of NF-KB. *J Biol Chem* 2002; **277**: 25203-25208

2 **Rosenthal EL,** Johnson TM, Allen ED, Apel IJ, Punturieri A, Weiss SJ. Role of the plasminogen activator matrix metalloproteinase systems in epidermal growth factor and scatter factor stimulated invasion of carcinoma cells. *Cancer Res* 1998; **58**: 5221-5230

3 **Yi S,** Chen JR, Viallet J, Schwall RH, Nakamura T, Tsao MS. Paracrine effects of hepatocyte growth factor/scatter factor on non-small-cell lung carcinoma cell lines. *Br J Cancer* 1998; **77**: 2162-2170

4 **Harvey P,** Clark IM, Jaurand MC, Warn RM, Edwards DR. Hepatocyte growth factor/scatter factor enhances the invasion of mesothelioma cell lines and the expression of matrix metalloproteinases. *Br J Cancer* 2000; **83**: 1147-1153

5 **Kundra V,** Anand-Apte B, Feig LA, Zetter BR. The chemotactic response to PDGF-BB: evidence of a role of ras. *J Cell Biol* 1995; **130**: 725-731

6 **Tamatani T,** Hattori K, Iyer A, Tamatani K, Oyasu R. Hepatocyte growth factor is an invasion/migration factor of rat urothelial carcinoma cells *in vitro*. *Carcinogenesis* 1999; **20**: 957- 962

7 **Li XN,** Ding YQ. HGF/SF-met signaling pathway and tumoral metastasis. *Medical Recapitulate* 2001; **7**: 643-644

8 **Weimar IS,** de Jong D, Muller EJ, Nakamura T, van Gorp JM, de Gast GC, Gerritsen WR. Hepatocyte growth factor/scatter factor promotes adhesion of lymphoma cells to extracellular matrix molecules via alpha4 beta 1 and alpha 5 beta 1 integrins. *Blood* 1997; **89**: 990-1000

9 **Fujisaki T,** Tanaka Y, Fujii K, Mine S, Saito K, Yamada S, Yamashita U, Irimura T, Eto S. CD44 stimulation induces integrin-mediated adhesion of colon cancer cell lines to endothelial cells by up-regulation of integrins and c-Met and activation of integrins and c-Met and activation of integrins. *Cancer Res* 1999; **59**: 4427-4434

10 **van der Voort R,** Taher TE, Wielenga VJ, Spaargaren M, Prevo R, Smit L, David G, Hartmann G, Gherardi E, Pals ST. Heparan sulfate-modified CD44promotes hepatocyte growth factor/scatter factor-induced signal transduction through the receptor tyrosine kinase c-Met. *J Biol Chem* 1999; **274**: 6499-6506

11 **Lenferink AE,** Busse D, Flanagan WM, Yakes FM, Arteaga CL. ErbB2/neu kinase modulates cellular p27(kip1) and cyclinD_1 through multiple signaling pathways. *Cancer Res* 2001; **61**: 6583-6591

12 **Donovan JC,** MilicA, Slingerland JM. Constitutive MEK/MAPK activation leads to p27(kip1) deregulation and antiestmgen resistance in human breast cancer cells. *J Biol Chem* 2001; **276**: 40888-40895

13 **Mao H,** Yuan AL, Zhao MF, Lai ZS. The effect of signal passway of mitogen activated protein kinase on hepatocellular carcinoma metastasis induced by VEGF. *Chin J Di* 2000; **20**: 14-16

14 **Feng S,** Song JD. Extracellular matrix degradational kinases in tumoral invasion and metastasis. *Zhongliu Fangzhi Yanjiu* 1999; **26**: 72-73

15 **Dinev D,** Jordan BW, Neufeld B, Lee JD, Lindemann D, Rapp UR, Ludwig S. Extracellular signal regulated kinase 5(ERK5) is required for the differentiation of muscle cells. *EMBO Rep* 2001; **2**: 829-834

16 **Testa JE,** Quigley JP. Principle and Practice of Cancer- Oncology (volume one). Translator: Xu CG, Zhang MH, Yang XJ. The Fifth Edition, Jinan: *Shandong Science and Technology Press* 2001: 146

17 **Yamamoto S,** Konishi I, Mandai M, Kuroda H, Komatsu T, Nanbu K, Sakahara H, Mori T. Expression of vascular endothelial growth factor (VEGF) in epithelial ovarian neoplasms: correlation with clinicopathology and patient survival, and analysis of serum VEGF levels. *Br J Cancer* 1997; **76**: 1221-1227

18 **Liotta LA,** Tryggvason K, Garbisa S, Hart I, Foltz CM, Shafie S. Metastatic potential correlates with enzymatic degradation of basement membrance collagen. *Nature* 1980; **284**: 67-68

19 **Kong LL,** Yang JW, Cui W. Progress of the relationship between matrix metalloproteinases and tumor. *J Jining Medical College* 2003; **26**: 64-66

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