The role of KDR in the interactions between human gastric carcinoma cell and vascular endothelial cell

Juan Ren, Lei Dong, Cang-Bao Xu, Bo-Rong Pan

INTRODUCTION
There exist many kinds of cells besides tumor cell in the solid neoplasm. The relations among all kinds of cells are very complicated. These cells depend on each other and contribute together to the genesis, development, invasion and metastasis of tumor. During the tumor angiogenesis and hematogenous metastasis, there exist complicated interactions between tumor cell (TC) and vascular endothelial cell (VEC). In the pre-angiogenesis, how does TC induce VEC to establish the tumor vascular system? How does TC influence the proliferation, degeneration, morphogenesis and functions of its neighbouring VEC? On the other hand, the interactions between the two cells play a role in the tumor hematogenous metastasis. There exist some complicated mechanisms in these processes. The study of tumor angiogenesis mainly focuses on the interactions among the vascular component cells while the study of tumor metastasis mainly focuses on the interactions between TC and its surrounding stroma. Seldom does anyone notice the interactions between TC and VEC. To better understand some mechanisms in gastric carcinoma angiogenesis and hematogenous metastases, we select human gastric carcinoma cell (HGCC) and human vascular endothelial cell (HVEC) to study their interrelations and some factors in these interactions [1]. Conditioned mediums (CMs) of HGCC and HVEC were prepared. The CM of one kind of cell was added to the other kind of cell, then the cell proliferation was measured by MTT method. Many studies have found that KDR, VEGF receptor 2, played an important role in regulating the biological functions of TC and VEC. In order to make clear the role of KDR in regulating the growth of HGCC and HVEC, antisense oligodeoxynucleotide (ASODN) specific to KDR mRNA was devised and was added acting on the two cells [2, 3]. There has been no one to devise KDR ASODN up to now. After the action of CM, the expression of KDR gene was detected. The purpose is to probe into the interactions between HGCC and HVEC and if KDR is involved in the interactions.

MATERIALS AND METHODS

Materials
Cell line HGCC line SGC7901 and HVEC line Eahy926 were employed. The expression of KDR on two cells was (+ +). KDR antisense oligodeoxynucleotide (ASODN) and the action of KDR ASODN ASODN, sense oligodeoxynucleotide (SODN) and mismatch oligodeoxynucleotide (MODN) specific to KDR mRNA were designed by the software “Primer 3”. The sequence of ASODN was: 5’CAC TTT GCT CGT CAT CCT G 3’, The sequence of SODN was 5’CAG GAT GCA GAG CAA GGT G 3’, The sequence of MODN was 5’CAC TTT GAT CTA CAC CCT G 3’. The way of KDR ASODN action: cells were placed in serum free medium for growth arresting. At different periods, KDR mRNA was arrested. At different periods, the proliferation of cell was measured by MTT. After the action of CM, the expression of KDR gene was detected. The purpose is to probe into the interactions between HGCC and HVEC and if KDR is involved in the interactions.
**Methods**

Preparing CM, measuring activity of conditioned medium (CM) and measuring level of hVEGF  

Cells in different confluent states were washed twice with PBS, then 3 mL culture medium was added to the cells. The medium what was taken as CM was collected after different periods. After the growth of HGCC and HVEC was arrested for 24 h and 6 h respectively, CM was added. The proliferation of cell was measured by MTT after different culturing periods. The cell without the action of CM was taken as control. The level of hVEGF in CMs of two kinds of cells was measured by ABC-ELISA kit (Jingmei, Beijing).

MTT (methyl tetrazolium colorimetry) 20 µL MTT solution (5 g/L) was added to 200 µL medium in each well of 96 well plate. 4 h later, the supernant was discarded, 150 µL DMSO (Dimethylsulphoxide) was added. After the crystal was dissolved completely, absorption spectrum was measured at 490 nm in the enzyme linked immunosorbent assay meter. The inhibitory rate of cell proliferation = \( \frac{1 - (\text{the mean A of experimental group})}{\text{the mean A of control group}} \times 100\% \)

**Detecting the expression of KDR gene**  

After the action of KDR ASODN or the action of CM of one kind of cell, the expression of KDR gene was measured by in situ hybridization for KDR mRNA level and immunohistochemical staining for KDR protein level. Probe for KDR mRNA was designed by the software "Primer 3". The sequence is 5’ GGT AGG AGA GGA TAT CCA GCC TG 3’; Probe labeling and in situ hybridization(ISH) were carried on according to the manuals of the Dig Oligodeoxynucleotide Tailing Kit and Dig Detection Kit (Boehringer Mannheim, Germany) respectively. PBS was substituted for anti-Dig-Ap as negative control. Immunohistochemical staining for KDR protein on the cells was carried on according to the manual of the SABC Kit (Huamei, Henang). KDR polyclonal antibody (Santa cruz, USA) was diluted 1:100. Secondary mouse-anti-rat antibody (Huamei, Henang) was diluted 1:25. PBS was substituted for primary antibody as negative control. The sections were analyzed for A value in the image analysis apparatus.

**Statistical analysis**  

\( t \) test was used to compare the means.

**RESULTS**

**Effects of KDR ASODN on the proliferation of HGCC and HVEC**  

KDR ASODN inhibited the proliferation of HGCC and HVEC significantly. It produced effects in 0.5-1 µmol/ L and 3-6 h later. The cell proliferation inhibitory rate could amount to more than 50 %. The inhibitory rate was related to the dose and action periods(Table 1, 2).

**Difference between the effects of KDR ASODN and SODN, MODN on HGCC, HVEC**  

There existed significant difference between the effects of KDR ASODN and SODN, MODN on the proliferation of HGCC and HVEC (Table 3).

**Effects of KDR ASODN on the expression of KDR gene in HGCC and HVEC**  

Through ISH for detecting KDR mRNA level and immunohistochemical staining for KDR protein level, it was found that KDR ASODN inhibited the expression of KDR gene in HGCC and HVEC significantly(Table 4) (Figure 1).

**Table 1**  

| KDR ASODN dose/ (µmol/ L) | HGCC Inhibitory rate of cell proliferation(%) | HVEC |
|---------------------------|---------------------------------------------|-------|
| (0(control))              | 0                                           | 0     |
| 0.5                       | 21.32<sup>a</sup>                          | 0     |
| 1                         | 28.31<sup>b</sup>                          | 15.33<sup>b</sup> |
| 5                         | 34.56<sup>b</sup>                          | 30.53<sup>b</sup> |
| 10                        | 45.59<sup>b</sup>                          | 39.67<sup>b</sup> |
| 15                        | 55.35<sup>b</sup>                          | 54.83<sup>b</sup> |
| 20                        | 50.74<sup>b</sup>                          | 48.79<sup>b</sup> |

<sup>a</sup>P<0.01 vs control.

**Table 2**  

| KDR ASODN action time/ h | HGCC Inhibitory rate of cell proliferation(%) | HVEC |
|--------------------------|---------------------------------------------|-------|
| (0(control))             | 0                                           | 0     |
| 3                        | 16.41<sup>b</sup>                          | 0     |
| 6                        | 18.99<sup>b</sup>                          | 6.67<sup>b</sup> |
| 12                       | 28.96<sup>b</sup>                          | 10.36<sup>b</sup> |
| 24                       | 38.90<sup>b</sup>                          | 37.52<sup>b</sup> |
| 48                       | 55.35<sup>b</sup>                          | 54.83<sup>b</sup> |
| 72                       | 50.45<sup>b</sup>                          | 46.18<sup>b</sup> |

<sup>b</sup>P<0.01 vs control.

**Table 3**  

| Types of KDR ODN | HGCC Inhibitory rate of cell proliferation(%) | HVEC |
|------------------|---------------------------------------------|-------|
| No ODN (control) | 0                                           | 0     |
| ASODN            | 45.07<sup>b</sup>                          | 31.18<sup>b</sup> |
| SODN             | 3.15<sup>b</sup>                           | 2.61<sup>b</sup> |
| MODN             | 2.88<sup>b</sup>                           | 2.02<sup>b</sup> |

<sup>b</sup>P<0.01, vs control.  

<sup>d</sup>P<0.01, vs ASODN group.

**Table 4**  

| A of HGCC | A of HVEC |
|-----------|-----------|
| mRNA      | protein   |
| Before(control) | 0.35±0.03 | 0.33±0.02 |
| After     | 0.16±0.02<sup>b</sup> | 0.15±0.02<sup>b</sup> |
| mRNA      | protein   |
| Before(control) | 0.37±0.03 | 0.34±0.03 |
| After     | 0.16±0.02<sup>b</sup> | 0.15±0.02<sup>b</sup> |

<sup>b</sup>P<0.01, vs control.
Figure 1  Expression of KDR gene in HGCC and HVEC before and after the action of KDR ASODN and CM
A. KDR mRNA in HGCC (Control) (in situ hybridization); B. KDR mRNA in HGCC after the action of KDR ASODN (in situ hybridization); C. KDR mRNA in HGCC after the action of CM of HVEC (in situ hybridization); D. KDR protein in HGCC (Control) (immunohistochemical staining); E. KDR protein in HGCC after the action of KDR ASODN (immunohistochemical staining); F. KDR protein in HGCC after the action of CM of HVEC (immunohistochemical staining); G. KDR mRNA in HVEC (Control) (in situ hybridization); H. KDR mRNA in HVEC after the action of KDR ASODN (in situ hybridization); I. KDR mRNA in HVEC after the action of CM of HGCC (in situ hybridization); J. KDR protein in HVEC (Control) (immunohistochemical staining); K. KDR protein in HVEC after the action of KDR ASODN (immunohistochemical staining); L. KDR protein in HVEC after the action of CM of HGCC (immunohistochemical staining)
Effect of conditioned medium of gastric carcinoma cell on vascular endothelial cell

The conditioned medium of HGCC could stimulate the proliferation of HVEC (\(P < 0.05\) vs no-CM action group) significantly. The stimulation effect was related to the CMs of different cell confluent state, different preparing periods, different volume fraction and different action periods (Table 5, 6).

**Table 5** Different dose-time-effects of CMs of subconfluent and confluent HGCC on HVEC (n=8, x±s)

| CM volume fraction | 100%CM | 80%CM | 50%CM | 30%CM | 10%CM |
|--------------------|--------|-------|-------|-------|-------|
| t/h cell confluent state | Subconfluent | Confluent | Subconfluent | Confluent | Subconfluent | Confluent | Subconfluent | Confluent | Subconfluent | Confluent |
| 24                  | 2.38±0.01a | 1.29±0.00a | 2.70±0.01a | 1.55±0.02a | 2.21±0.01a | 1.49±0.02a | 2.10±0.03a | 1.44±0.01ab | 1.92±0.02a | 1.11±0.01ab |
| 48                  | 1.60±0.01a | 1.22±0.01a | 1.54±0.01a | 1.33±0.02a | 1.38±0.02a | 1.23±0.02a | 1.37±0.02a | 1.21±0.01ab | 1.31±0.01a | 1.15±0.02ab |
| 72                  | 0.89±0.00  | 0.85±0.00  | 0.99±0.01  | 1.03±0.01  | 1.07±0.01  | 1.11±0.01  | 1.27±0.01  | 1.01±0.00  | 1.01±0.00  | 1.15±0.01  |

\(^{a}P<0.05\) vs No-CM action group, \(^{b}P<0.05\) vs subconfluent group.

**Table 6** Different dose-time-effect of CMs of different preparing periods of confluent HGCC on HVEC (n=8, x±s)

| CM volume fraction | 100%CM | 80%CM | 50%CM | 30%CM | 10%CM |
|--------------------|--------|-------|-------|-------|-------|
| t/h Preparing periods | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM | 24hCM | 48hCM |
| 24                  | 1.29±0.01a | 1.38±0.01a | 1.55±0.02a | 1.39±0.01a | 1.49±0.01a | 1.38±0.01a | 1.44±0.02a | 1.30±0.01a | 1.11±0.01a | 1.00±0.01a |
| 48                  | 1.22±0.01a | 1.11±0.02a | 1.33±0.01a | 1.14±0.02a | 1.23±0.01a | 1.03±0.01a | 1.21±0.01a | 1.03±0.01a | 1.15±0.01a | 1.02±0.01a |
| 72                  | 0.85±0.01  | 0.78±0.00  | 0.99±0.01  | 0.92±0.00  | 1.07±0.02b | 1.14±0.01b | 1.27±0.01b | 1.20±0.01b | 1.15±0.01b | 1.16±0.01b |

\(^{a}P<0.05\) vs No-CM action group, \(^{b}P<0.05\) vs preparing for 24 h CM group.

**Effect of conditioned medium of vascular endothelial cell on gastric carcinoma cell**

The conditioned medium of HVEC could inhibit the proliferation of HGCC (\(^{a}P<0.05\) vs no-CM action group) significantly. The inhibitory effect was related to the different cell confluent states and different volume fractions (Table 7).

**Table 7** Effects of CMs of subconfluent and confluent HVEC on HGCC for 48 h (n=8, x±s)

| Volume fraction | CM of subconfluent HGCC | CM of confluent HGCC |
|-----------------|------------------------|---------------------|
| 100%CM          | 52.97±0.01a            | 31.62±0.02ab        |
| 80%CM           | 54.26±0.01a            | 30.46±0.01ab        |
| 50%CM           | 23.46±0.01b            | 19.00±0.01ab        |
| 30%CM           | 21.70±0.00             | 2.13±0.01b          |
| 10%CM           | 14.36±0.00             | 1.61±0.00b          |

\(^{a}P<0.05\) vs No-CM action group; \(^{b}P<0.05\) vs subconfluent group

**Expression of KDR gene in HVEC before and after the action of HGCC CM**

The mRNA level (A value of in situ hybridization) before and after the action of HGCC CM was (0.37±0.03), (0.48±0.01b) respectively (\(^{a}P<0.01\) vs before the action). The protein level (A value of immunohistochemical staining) before and after the action of HGCC CM was (0.34±0.03), (0.48±0.02b) respectively (\(^{a}P<0.01\) vs before the action). So, after the CM of HGCC acted on HVEC, the expression level of KDR gene in HGCC was increased significantly (Figure 1).

**Expression of KDR gene in HGCC before and after the action of HVEC CM**

The mRNA level (A value of in situ hybridization) before and after the action of HVEC CM was (0.35±0.03), (0.22±0.02b) respectively. The protein level (A value of immunohistochemical staining) before and after the action of HVEC CM was (0.33±0.03), (0.23±0.02b) respectively. So, after the CM of HVEC acted on HGCC, the expression level of KDR gene in HGCC was inhibited significantly (Figure 1).

**The hVEGF levels in the CMs of HGCC and HVEC**

The hVEGF level in the CMs of HGCC and HVEC was (92.06±1.69 ng/L), (77.70±8.04 ng/L) respectively.

**DISCUSSION**

An important advance in oncology is that the importance of the tumor angiogenesis in the tumor genesis, growth and metastases, and the importance of the vascular targeting therapy in the tumor treatment have been proved. One of the most important factors in the tumor angiogenesis is vascular endothelium growth factor (VEGF). VEGF can promote and maintain the establishment of tumor vascular system. And it can promote the tumor growth directly. VEGF can induce the mitogenesis and chemotaxis of vascular endothelial cell (VEC)
and tumor cell (TC) intensely. Almost all types of TC and tumor VEC can secret VEGF. But the expression of VEGF in the normal tissue is very low. In the four VEGF receptors, R2; KDR is the main receptor which gives play to VEGF functions, while other receptors play little role in cell growth. KDR is highly expressed on the TC and tumor VEC while weakly expressed on the normal tissues. In order to make clear the role of KDR in regulating the growth of HGCC and HVEC. We devised the antisense oligodeoxynucleotide (ASODN) specific to KDR mRNA. There has been no one to devise KDR ASODN up to now. The results showed that KDR ASODN could inhibit the growth and expression of KDR gene of HGCC and HVEC significantly. There was a great difference between the effects of ASODN and SODN, MODN. The CMs of two cells had a certain level of hVEGF. This illustrated that KDR ASODN could really enter into the cells and specifically block the expression of KDR gene to interrupt the selfsecretion and paracrine growth-stimulation pathway of VEGF. The results showed that VEGF and its receptor KDR played a key role in regulating the proliferation of HGCC and HVEC.

In the human solid neoplasm, there exist many kinds of cells besides the tumor cells, such as: interstitial cell, immunocyte and vascular cell. They depend on each other to contribute to the tumor genesis, development, invasion and metastasis. The interactions among them are complicated. During the tumor angiogenesis and tumor hematogenous metastasis, both VEC and TC contribute to finishing the pathology processes. The interactions between them act in close coordination to establishing tumor vascular system and finishing hematogenous metastasis. There exist very complicated interrelations between TC and VEC. In the pre-angiogenesis, how does TC induce VEC to establish the tumor vascular system? That is, how does TC influence the proliferation, degeneration, morphogenesis and functions of its neighboring VEC? Or, how does VEC influence these characters of its neighboring TC? These are all unclear. On the other hand, the interactions between two cells are involved in the tumor infiltration and hematogenous metastasis. Tumor vascular provides the passage for tumor infiltration and metastasis. The integrated vascular endothelium is the barrier to the tumor infiltration and metastasis. How do these two kinds of cells interact reciprocally to render the tumor cell to adhere to and destroy the vascular endothelium to enter vascular lumen and damage it again to enter stroma? There exist some complicated mechanisms in this process. The study of tumor angiogenesis mainly focuses on the interactions among the vascular component cells while the study of tumor invasion and metastasis mainly focuses on the interactions between TC and surrounding stroma. Seldom has anyone noticed the interactions between TC and VEC. The relations between TC and VEC are not clear. Studies on this aspect are very few. Studies abroad always choose melanoma, glioblastoma, cephalo-cervical squamous carcinoma and hepatocellular carcinoma [14-19], but not gastric carcinoma as the target research yet. And the results are also controversial. We have not found anyone who studies gastric carcinoma yet. There are lots of studies on gastric carcinoma [20-28], but seldom in this aspect. To better understand some mechanisms in gastric carcinoma angiogenesis and hematogenous metastases, we select HGCC and HVEC to study their interrelations and the mechanisms. Considering the results showed that KDR was an important regulator to the growth of HGCC and HVEC, we study the role of KDR in the interactions between the two cells.

Results showed that CMs of HGCC with different confluent state, different preparing periods, different volume fraction and different action periods stimulated the growth of HVEC significantly. The activity of CM of subconfluent cell was stronger than that of CM of confluent cell. The activity of CM preparing for 24 h was stronger than that of CM preparing for 48 h. After the nutrition exhaustion was replenished, the more of the volume fraction, the stronger of the activity. This is consistent with the results of some studies. Someone found CM of bladder carcinoma stimulated the growth of HVEC. Others found that cephalo-cervical squamous carcinoma cell stimulated the growth of HVEC through secreting FGF and VEGF. But there were other contrary viewpoints. Zhao found bladder carcinoma cell inhibited the growth of HVEC through a 10-16bp RNA fragment. Albini found some kinds of tumor cells inhibited HVEC to form vascular through secreting IFN-γ. There was also a neutral objection: TC has little effect on the proliferation of HVEC. Some researchers found that although TC had no effect on the growth of HVEC, TC could change morphology of HVEC or its sensitivity to TNF-α. We think these different results are due to different cell types. Results showed that HGCC could secret a certain level of hVEGF. CM of HGCC could up-regulate the expression of KDR gene in HVEC. It illustrated that KDR played an important role in the growth-stimulation of HGCC to HVEC. It needs further study to show if there exist other factors and mechanisms involved in this effect of HGCC on HVEC.

Our results also showed that CM of HVEC could significantly inhibit the growth of HGCC. The activity of CM of subconfluent cell was stronger than that of CM of confluent cell. After the nutrition exhaustion was replenished, the more of the volume fraction, the stronger of the activity. We do not know what this inhibition means exactly. Maybe in the tumor angiogenesis, the inhibition could prevent HGCC to occupy the place of vascular or participate into the vascularization. Then the stimulation of vascular system to growth of gastric carcinoma is not through the direct interaction between HGCC and HVEC, but the establishment of the vascular system passes nutrition to HGCC and excretes its metabolism waste. HVEC also secreted a certain level of hVEGF and VEGF could stimulate the proliferation of HGCC. But CM of HVEC inhibited the growth of HGCC and the KDR gene expression in HGCC. Although HVEC produced growth-stimulator, VEGF, HVEC could interrupt the role of VEGF on HGCC through reducing the level of its main functional receptor. KDR. It illustrated that KDR play an important role in the growth-inhibition of HVEC to HGCC. Whether HVEC secretes some other growth-inhibiting factors to inhibit the proliferation of HGCC or not needs further study.

REFERENCES
1 Ren J, Dong L, Xu CB, Pan BR, Li M Z. Interactions between the human gastric carcinoma cell and vascular endothelial cell. Shijie He 2001; 9: 1254-1260
2 Ren J, Dong L, Xu CB. Effect of KDR antisense oligodeoxynucleotide on the human gastric carcinoma cell. Di- si Junyi Daxue Xuebao 2002; 23: 333-336
3 Ren J, Dong L, Xu CB. Effect of KDR ASODN on the human vascular endothelial cell. Jichu Yixue Y Wu Liu 2002; 8: in press.
4 Von BC, Hayen W, Hartmann A, Mueller KW, Alloizio B, Nehls V. Endothelial capillaries chemotactically attract tumor cells. J Pathol 2001; 193: 267-276
5 Okamoto H, Ohigashi H, Nakamori S, Ishikawa O, Imaoka S, Mukai M, Kusama T, Fuji H. Reciprocal functions of liver tumor cells and endothelial cells. Involvement of endothelial cell migration and tumor cell proliferation at a primary site in distant metastasis. Eur Surg Res 2000; 32: 374-379
6 Moreno A, Villar ML, Camara C, Luque R, Cespon C, Gonzalez-PP, Roy G, Lopez JJ. Interleukin-6 dimers produced by endothelial cells inhibit apoptosis of B-chroniclymphocytic leukemia cells. Blood 2001; 97:242-249

7 Brandvold KA, Neman P, Ruddell A. Angiogenesis is an early event in the generation of myc-induced lymphomas. 0 nogene 2000; 19: 2780-2785

8 Dedeos, Rosati S, Jacobs S, Kamps WA, Vellenga E. Increased bone marrow vascularization in patients with acute myeloid leukemia is a possible role for vascular endothelial growth factor. Br J Haematol 2001; 113:296-304

9 Hewett PW. Identification of tumor-induced changes in endothelial cell surface protein expression in vitro model. Int J Biochem Cell Biol 2001; 33:325-335

10 Luo J, Guo P, Matsuda K, Truong N, Lee A, Chun C, Cheng SY, Kore M. Pancreatic cancer cell-derived vascular endothelial growth factor is biologically active in vitro and enhances tumorigenicity in vivo. Int J Cancer 2001;92:361-369

11 Witt J, Gabel SP, Meisenger J. Interrelationship between protein phosphatase-2A and cytokoskeletal architecture during the endothelial cell response to soluble products produced by human head and neck cancer. Otolaryng Head Neck Surg 2000;122:721-727

12 Kuroda K, Miyata K, Tsutsumi Y. Preferential activity of wild-type and mutant tumor necrosis factor-α against tumor-derived endothelial like cells. Jpn J Cancer Res 2000; 91:59-67

13 Shemirani B. Head and Neck squamous cell carcinoma lines produce biologically active angiogenic factors. Oral Oncol 2000; 36: 61-66

14 Beierle EA, Strander LF, Berger AC, Chen MK. VEGF is upregulated in a neuroblastoma and hepatocyte coculture model. J Surg Res 2003; 97:344-40

15 Vidal Vanaclocha F, Fantuzzi G, Mendoza L. IL-18 regulated IL-1 dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci USA 1999; 97:734-739

16 Aoki M, Kanamori M, Yudoh K, Ohmori K, Yasuda T, Kimura T. Effects of vascular endothelial growth factor and Flk-1 expression on angiogenesis in the murine metastatic RCT carcinoma. Tumour Biol 2001;22:239-246

17 Liu W, Davis DW, Ramirez K, McConkey DJ, Ellis LM. Endothelial cell apoptosis is inhibited by a soluble factor secreted by human colon cancer cells. Int J Cancer 2001; 92:26-30

18 Albinelli A, Marchione C, Del Grosso F. Inhibition of angiogenesis and vascular tumour growth by interferon-pro-

19 Kuroda K, Miyata K, Tsutsumi Y. Preferential activity of wild-type and mutant tumor necrosis factor-α against tumor-derived endothelial like cells. Jpn J Cancer Res 2000; 91:59-67

20 Wux K, Zhao Y, Liu BH, Li Y, Liu F, Guo JY, Yu WP. RRR-α-tocopheryl succinate inhibits human gastric cancer SGC-7901 cell growth by inducing apoptosis and DNA synthesis arrest. World J Gastroenterol 2002;8:26-30

21 Wang X, Lan M, Shi YQ, Lu J, Zhong YX, Wu HP, Zai HH, Ding J, Wu KC, Pan BR, Jin JP, Fan DM. Differential display of vcinrastine resistance-related genes in gastric cancer SGC7901 cell. World J Gastroenterol 2002;8:54-59

22 Yao XL, Xu B, Song YQ, Zhang WD. Overexpression of cyclin E1 in MKN45 gastric cells with Helicobacter pylori-induced gastric precancer. World J Gastroenterol 2002;8:60-63

23 Yang SM, Fang DC, Luo YH, Lu R, Liu WW. Effect of antisense gene to human KLK5 and VEGF expression on trophoblast cell invasion and expression of apo-PTOS associated gene. Shijie Huanren Xiaohua Zazhi 2002;10:149-152

24 Fawcett P, Stawski G, Huang Y, Wang MX, Sun KL. E-cadherin gene mutation in precancerous condition, early and advanced stage of gastric cancer. Shijie Huanren Xiaohua Zazhi 2002;10:153-156

25 Wang W, Luo HS, Yu BP. Expression of human telomerase reverse transcriptase gene and c-myc protein in gastric carcinogenesis. Shijie Huanren Xiaohua Zazhi 2002;10:258-261

26 Li JY, Yu JP, Luo HS, Yu BP, Huang JA. Effects of nonsteroidal anti-inflammatory drugs on the proliferation and cyclooxygenase activity of gastric cancer cell line SGC 7901. Shijie Huanren Xiaohua Zazhi 2002;10:262-265

27 Xue FB, Xu YY, Wan Y, Pan BR, Ren J, Fan DM. Association of H. pylori infection with gastric carcinoma a Meta analysis. World J Gastroenterol 2001;7:801-804

28 Liu DH, Zhang XY, Fan DM, Huang YQ, Zhang JS, Huang WQ, Zhang YQ, Huang QS, Ma WY, Chai YB, Jin M. Expression of vascular endothelial growth factor and its role in oncogenesis of human gastric carcinoma. World J Gastroenterol 2001;7:500-505

29 Cain L, Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: A case-control study. World J Gastroenterol 2001;7:506-509

30 He KS, Su Q, Chen ZC, He XT, Long ZF, Ling H, Zhang LR. Expression, deletion and mutation of p16 gene in human gastric cancer. World J Gastroenterol 2001;7:515-521

31 Fang DC, Yang SM, Zhou XD, Wang DX, Luo YH. Telomere erosion is independent of microsatellite instability but related to loss of heterozygosity in gastric cancer. World J Gastroenterol 2001;7:522-526

32 Cui JH, Krueger U, Henne-Bruns D, Kremer B, Kalthoff H. Orthotopic transplantation model of human gastric intestinal cancer and detection of micrometastases. World J Gastroenterol 2001;7:381-386

33 Ni CR. Expression significance of GST-π, P-gp, Top II and nm23H1 in gastric carcinoma. Shijie Huanren Xiaohua Zazhi 1999: 897-901.

34 Wang X, Zhao YQ, Fan DM. Construction and expression of gastric cancer MGF mimic epitope fused to heat shock protein 70. Shijie Huanren Xiaohua Zazhi 2001; 9:892-896

35 Liu DH, Zhang W, Su YP, Zhang XY, Huang YX. Construction of eukaryotic expression vector of sense and antisense VEGF-165 and its expression regulation. Shijie Huanren Xiaohua Zazhi 2001; 9:886-891

36 Liu WC, Mu HX, Ren J, Zhang XY, Pan BR. Anti tumor activity of defensin on gastric cancer cell line in vitro. Shijie Huanren Xiaohua Zazhi 1999:862-866

37 Han Y, Shi YQ, Zhang Y, Nie YZ, Zhang HB, Zhang ML, Pan BR, Fan DM. Protein kinase C is related to multidrug resistance induced by Mdr1Ag. Shijie Huanren Xiaohua Zazhi 2001; 9:517-521

38 Kong XD, Zhang SZ, Hu JK, Xiao CY, Sun Y, Xia QJ. Abnormalities of p15 gene and protein expression in gastric cancers. Shijie Huanren Xiaohua Zazhi 2001; 9:513-518

39 Yang XX, Yin L, Zhang JS, Bai WW, Li YM, Sun ZC. hTERT expression and cellular immunity in gastric cancer and precancer. Shijie Huanren Xiaohua Zazhi 2001; 9:508-512

40 Chen SY, Wang JY, Ji Y, Zhang XD, Zhu CW. Effects of Helicobacter pylori and protein kinase C on gene mutation in gastric cancer and precancerous lesions. Shijie Huanren Xiaohua Zazhi 2001; 9:302-307

41 Cheng SD, Wu YL, Zhang YP, Qiao MM, Guo QS. Abnormal drug accumulation in multidrug resistant gastric carcinoma cells. Shijie Huanren Xiaohua Zazhi 2001; 9:131-134

42 Wang B, Shi LC, Zhang WB, Xiao CM, Wu JF, Dong YM. Expression and significance of p16 gene in gastric cancer and its precancerous lesions. Shijie Huanren Xiaohua Zazhi 2001; 9:39-44

43 Liu HF, Liu WW, Fang DC. Effect of combined anti Fas mAb and IFN-γ on the induction of apoptosis in human gastric carcinoma cell line SGC-7901. Shijie Huanren Xiaohua Zazhi 2000; 8:1361-1364

44 Gao MX, Zhang NZ, Ji CX. Estrogen receptor and PCNA in gastric carcinomas. Shijie Huanren Xiaohua Zazhi 2000; 8:1117-1120

45 Xia JZ, Qiu ZG, Liu BY, Yan M, Yin HR. Significance of immunohistochemically demonstrated micrometastases to lymph nodes in gastric carcinomas. Shijie Huanren Xiaohua Zazhi 2000; 8:1113-1116

Edited by Pang LH