Online Submissions: wjg.wjgnet.com
www.wjgnet.com
wjg@wjgnet.com

World J Gastroenterol 2008 January 14; 14(2): 180-184
World Journal of Gastroenterology ISSN 1007-9327

REVIEW

Adenoviral gene therapy in gastric cancer: A review

Nima Khalighinejad, Hesammodin Hariri, Omid Behnamfar, Arash Yousefi, Amir Momeni

Nima Khalighinejad, Hesammodin Hariri, Omid Behnamfar, Arash Yousefi, Amir Momeni, Isfahan University of Medical Sciences, Khalighinejad Nima, Number 111, 5th street, Mardavij Street, Isfahan, Iran
Correspondence to: Khalighinejad Nima, Isfahan University of Medical Sciences, Number 111, 5th street, Mardavij Street, Isfahan, Iran. nima_kh8@yahoo.com
Telephone: +98-311-6683637 Fax: +98-311-6681399
Received: July 21, 2007 Revised: October 24, 2007

Abstract

Gastric cancer is one of the most common malignancies worldwide. With current therapeutic approaches the prognosis of gastric cancer is very poor, as gastric cancer accounts for the second most common cause of death in cancer related deaths. Gastric cancer like almost all other cancers has a molecular genetic basis which relies on disruption in normal cellular regulatory mechanisms regarding cell growth, apoptosis and cell division. Thus novel therapeutic approaches such as gene therapy promise to become the alternative choice of treatment in gastric cancer. In gene therapy, suicide genes, tumor suppressor genes and anti-angiogenesis genes among many others are introduced to cancer cells *via* vectors. Some of the vectors widely used in gene therapy are Adenoviral vectors. This review provides an update of the new developments in adenoviral cancer gene therapy including strategies for inducing apoptosis, inhibiting metastasis and targeting the cancer cells.

Key words: Gastric cancer; Adenovirus; Gene therapy; Vector; Apoptosis; Metastasis

http://dx.doi.org/10.3748/wjg.14.180

Khalighinejad N, Hariri H, Behnamfar O, Yousefi A, Momeni A. Adenoviral gene therapy in gastric cancer: A review. *World J Gastroenterol* 2008; 14(2): 180-184

http://www.wjgnet.com/1007-9327/14/180.asp

INTRODUCTION

Gastric cancer is one of the most common malignancies in the world with an estimated 934 000 cases reported globally in 2002[1], and the second most common cause of death from cancer. The prognosis of gastric cancer is poor with an estimated relative 5 years survival rate of less than 20%[2].

In Iran, 26.1% of cancers reported in 2002 in men and 11.1% of cancers in women were due to gastric cancer. Gastric cancer is the first leading cause of Cancer related death in Iranian men and the second in Iranian females. The incidence rates of gastric cancer in Iran are well above the world average; it is the fourth common cancer in the world however it is the most common malignancy in Iran[3]. Comparing gastric cancer rates with the data of 30 years ago, shows that the overall incidence of gastric cancer in Iran has gradually increased over the years[4]. This is in contrary to the global trend which has seen a steady decrease in the incidence and mortality rates for gastric cancer.

The current best approach for treating gastric cancer is complete surgical removal of the tumor with the adjacent lymph nodes however the efficacy of this therapeutic approach as well as hormone, radio and chemotherapy is very limited. Thus new therapeutic approaches are needed[5,6].

Gastric cancer is a genetic disease developing from a multi-step process[7]. Single or multiple mutations in genes related to growth control, apoptosis, invasion and metastasis form the molecular genetic basis of malignant transformation and tumor progression[8], so a better understanding of the molecular basis of tumor host interactions leads to significant progress in the development of new therapeutic agents. Based on this theory, cancers can be curable if the regulating mechanisms are reintroduced to the cancer cells; this can be achieved using gene therapy, which is an emerging alternative for treatment of cancers[9,10].

In gene therapy vectors are used to insert new genes into cells, or to switch off existing genes[11]. Some of the most efficient vectors used for gene therapy are viral vectors, as they naturally insert their genes into cells, they are well adopted and equipped for such a task. In recent years adenoviruses have been extensively used as vectors to deliver foreign genome into mammalian cells. Adenoviruses have certain features, which make them attractive vectors for gene transfer to target cells. Some of these characteristics include their ability to infect a broad range of cell types, including dividing as well as non-dividing cells, the ease with which adenovirus genome can be manipulated, and the ability to obtain high titers. To achieve an adenoviral vector, foreign cDNAs are inserted into the adenovirus genome, resulting in recombinant adenoviruses containing the gene of choice.

Cancer gene therapy has 3 essential goals: the first is

to suppress cancer growth and induce apoptosis in cancer cells, the second goal is to inhibit metastasis of malignancy to other sites and finally the effects of gene therapy must be limited to cancer cells. These goals can be achieved using some strategies, which we will further explain below[12-15].

TUMOR SUPPRESSOR GENES AND APOPTOSIS INDUCING GENES

As mentioned earlier, one of the ways for stopping cancer growth is to reintroduce the regulating mechanisms into cells, these mechanisms are supervised in the first place by tumor suppressor genes, so by inserting these genes into cells, tumor growth can be suppressed. Reintroduction of tumor suppressor genes also has the additional advantage of making targeted cells susceptible to chemotherapy[16].

The most famous tumor suppressor gene is P53, which is mutated in 60% of gastric cancers and in almost all other cancers[17]. Introduction of the p53 gene *via* a recombinant adenovirus has been shown to inhibit the growth of gastric cancer cells with mutated p53 *in vitro* and *in vivo*[18,19]. P53 not only regulates cell cycle and cell growth but it also has a crucial role in activating proapoptotic genes such as Bax, Apaf-1, Fas and PTEN thus not only limiting cancer growth but also inducing apoptosis in cancer cells (if the proapoptotic genes are not mutated themselves). Other tumor suppressor genes useful in gastric cancer include: P16 gene and Fhit[20-23].

There are two approaches for inducing apoptosis in cancer cell lines, the first is to introduce native proapoptotic genes into cancer cells, this has the additional advantage of minimum toxicity of novel gene for non cancer cells (which already have a copy of native proapoptotic genes). The second approach on the other hand is to introduce genes of non-mammalian enzymes into cancer cells, so that they can convert non toxic prodrugs into highly toxic substances that will kill the cell[24]. This approach has the advantage of bystander effect, which means not only the infected cell will be killed but it will release the highly toxic drug which will kill the adjacent cells as well[25].

Among genes introduced into cells to induce apoptosis, Bax is a good example, as it independently of cell's P53 activity can make the cell commit suicide; it also has the further advantage of sensitizing the malignant cells to conventional anti-tumor treatments[26]. Some other genes that can be used in inducing apoptosis in gastric cancer cells include genes, which are effective in Caspase 3 common pathway including Caspase 8[27,28].

Another method of killing cancer cells is to transfect them with genes of non-mammalian enzymes which can convert non toxic prodrugs into highly toxic agents, destroying the infected cell and nearby cells. The most widely used suicide gene/prodrug system is the herpes simplex virus (HSV) thymidine kinase (HSV-tk)/ ganciclovir (GCV) system that can convert the prodrug GCV into phosphorylated GCV. The phosphorylated GCV inhibits cellular DNA synthesis and leads to the killing of cancer cells *via* apoptotic and non-apoptotic mechanisms[29,30]. A similar approach involves the expression of recombinant *E. coli* cytosine deaminase (CD) in gastric cancer cells together with the administration of 5-fluorocytosine (5-FC). 5-FC is given orally and converted to 5-fluorouracil in the tumor cells expressing CD[31] (Table 1).

Table 1 A review of recent methods used in gastric cancer adenoviral gene therapy

Apoptosis inducers & tumor suppressors	
Gene	**Conclusion**
FasL	Infection of human gastric carcinoma cells (SGC-7901) with Ad-FasL showed increased expression of FASL, resulting in apoptosis[32].
HDAC inhibitor	Induces apoptosis in cancer cells expressing wild and pseudo-wild type p53 *via* activating the p53 through acetylation[35].
E2F-1	E2F -1 is a transcription factor that regulates cell cycle progression into S-phase. Combining E2F-1 overexpression with cyclin-dependent kinase inhibitors results in an enhanced apoptotic response, causing nearly complete gastric tumor cell death[33].
Bax	In a study, Ad/Bax made marked Bax protein expression and effective apoptosis in MKN-1, MKN-7, MKN-28 gastric cell lines[34].
p51 and p53	p51 (p73L/p63/p40/KET), a p53 homologue, binds to p53-responsive elements to upregulate some p53 target genes and has been suggested to share partially overlapping functions with p53. p53 is an apoptosis promoting gene which could be used to eliminate tumor growth in gastric cancer. Adenovirally transduced p51A cDNA into human gastric cancer cells promotes apoptosis just like p53[36].
Caspase 8	Adv-Caspase 8 can selectively induce apoptosis in detached carcinoma cells[37].

ANTI ANGIOGENESIS GENES

Angiogenesis is essential for tumors in order to grow and form metastases. It is a multi step process, which is initiated by release of growth factors from cells, these growth factors include growth factors such as the vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and hepatocyte growth factor (HGF)[38-40]. Inhibiting angiogenesis in tumors can in theory stop a tumor from functioning and growing, it is especially useful in limiting the dissemination of cancer[41-43]. Anti angiogenesis treatment do not need to specifically target cancer cells to inhibit metastasis as creating an anti angiogenesis environment around the cancer is equally effective. An example of anti angiogenesis genes is NK4, an HGF antagonist. It has been proved that transfecting gastric cancer cells with NK4 can stop the formation of Intra tumor vessels as well as peritoneal metastasis[44]. Another anti angiogenesis gene which can be introduced into cells using adenoviruses is soluble VEGF receptor (sFlt1) (Table 2).

TARGETED GENE THERAPY

One of the challenges of current gene therapy vector development, concerns targeting a therapeutic gene

Table 2 A review of recent methods used in gastric cancer adenoviral gene therapy

Metastasis inhibitors	
Gene	**Conclusion**
NK4	Peritoneal tumors in mice treated early with adenoviral mediated NK4 were significantly reduced. In another study Ad.CMV.NK4 resulted in efficient production and secretion of NK4 and significantly inhibited proliferation, migration and invasion of gastric cancer cells[45].
Flt-1	When administered intra peritoneally it markedly reduced the number of metastatic nodules larger than 1 mm in diameter on the peritoneal surface[46].
Caspase 8	In a study it was determined that Caspase 8 can augment anoikis in MKN-45 cells and suppress its peritoneal dissemination in nude mice with xenograft gastric cancer transplants and consequently increase survival, Caspase 8 has also proved to be useful in limiting metastasis in other carcinomas originating from epithelial tissues[37].

Table 3 A review of recent methods used in gastric cancer adenoviral gene therapy

Targeted gene therapy	
Method	**Conclusion**
HDAC inhibitor	Increase expression of the Coxackie adenovirus receptor and subsequent transfection efficiency of the adenovirus in cancer cells[35].
COX-2	COX-2 promoter shows the strongest cytocidal effect in gastric cancer cells when it is used in a conditionally replicating adenovirus (CRAD) context & with adenoviral vectors displaying 5/3 chimeric fibers[56].
EPCAM	It has been demonstrated that there is a marked difference in expression of the human epithelial cell adhesion molecule (EpCAM) between normal and (pre)malignant lesions of the stomach and esophagus. Based on this, using EpCAM to achieve gastric and esophageal adenocarcinoma selective gene transfer.may be a feasible choice for cancer-specific gene therapy[57]. In a study using EPCAM antibody adhered to adenovirus, transduction of normal gastric epithelium and liver tissue was reduced at least 10-fold in comparison with native adenovirus however tumor transduction levels remained the same[58].
Carcinoembryonic antigen (CEA)	A remarkable degree of targeted gene delivery to gastric cancer cells was obtained with Adv-FZ33 with the fully human anti-carcinoembryonic antigen (CEA) monoclonal antibody, C2-45[59-62].

to diseased cells with the aim of achieving sufficient gene expression in the affected tissue, while minimizing toxicity and expression in other tissues[47,48]. The use of recombinant adenoviruses as vectors for gene therapy is restricted by the widespread distribution of the coxsackie and adenovirus receptor (CAR), which allows infection of a range of tissues and precludes specific *in vivo* targeting[49-51].

Targeting can be achieved at the level of capsid binding or at later transduction events by the use of tissue-specific promoters. Targeting at the level of binding is preferred because even the interaction of cells with empty capsids leads to toxic effects. However a combination of both strategies has its obvious advantages.

Manipulating capsid binding can be achieved by direct genetic modifications of the capsid proteins, or it is possible to add ligands to the capsid *via* polymers like polyethylene glycol (PEG) and poly-[N-(2-hydroxypropyl) methacrylamide] (HPMA). Using these approaches, the native tropism of the virus is ablated either by the addition of polymer to fiber knob or the use of an anti fiber neutralizing antibody in the context of a bi-functional conjugate and creating targeted tropism by adding ligands. For example, for targeting ovarian cancer cells, it is possible to attach FGF to PEG bonded to viral capsid thus increasing specificity and also efficiency of transfection.

Targeting can also be achieved using tissue-specific promoters (TSP)[52-54], for example in alpha-fetoprotein (AFP)-producing gastric tumors, the adenovirus-mediated expression of HSV-tk by an AFP enhancer/promoter element selectively eliminates AFP positive, but not AFP-negative cell lines when treated with ganciclovir[55] (Table 3).

CONCLUSION

During the past 10 years, much has been learnt about molecular alterations in gastric cancer. Based on the understanding of the molecular mechanisms underlying gastric carcinogenesis, new therapeutic strategies targeted at the molecular defects in the tumor cells have been designed and many promising therapy results have been obtained from *in vitro* or *in vivo* studies. Among these approaches, Adenoviral gene therapy has proven to be the most promising however the efficiency of gene therapy for treatment of cancer in humans has remained low, this may be due to low rates of transduction and specificity. There may be many reasons for this, but it is widely agreed that this is mainly due to the relative resistance of cancer cells to introduce foreign material combined with low transgene expression *in vivo*. One of the most important issues affecting the possible clinical application of gene therapy is the need to ensure the highest possible safety levels developing protocols and targeting adenoviral vectors for gastric cell lines may yield the answer to this problem. Another prospect worth mentioning is the benefit of combining several approaches in order to achieve the highest possible therapeutic effect, examples of combined therapy approaches include combining Ad-p53 with HDAC inhibitor and sodium butyrate (SB), this has shown a significantly higher growth suppressive effect than single treatments of each. Another example shows combining NK4 with Cysplatin has enhanced inhibitory effect on peritoneal metastasis, suggesting that the combination of intra peritoneally chemotherapy with NK4 might be effective[63,64].

Recent studies have shed light on pathogenesis of gastric cancer, it has been long argued that *H Pylori* acts as a carcinogenic factor in inducing gastric adenocarcinoma, recent studies have supported this hypothesis, thus an area of future investigation in treatment of gastric cancer could involve *H Pylori* modification and also the intracellular

pathways altered by *H Pylori* in gastric mucosa. Although it has been reported that simple eradication of *H Pylori* without reversing the effects *H Pylori* has caused on gastric mucosa has limited efficacy in preventing gastric cancer, however there is a need for further investigation in this field[65-67].

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108
2 **Neugut AI**, Hayek M, Howe G. Epidemiology of gastric cancer. *Semin Oncol* 1996; **23**: 281-291
3 **Sadjadi A**, Nouraie M, Mohagheghi MA, Mousavi-Jarrahi A, Malekezadeh R, Parkin DM. Cancer occurrence in Iran in 2002, an international perspective. *Asian Pac J Cancer Prev* 2005; **6**: 359-363
4 **Yazdizadeh B**, Jarrahi AM, Mortazavi H, Mohagheghi MA, Tahmasebi S, Nahvijo A. Time trends in the occurrence of major GI cancers in Iran. *Asian Pac J Cancer Prev* 2005; **6**: 130-134
5 **Hill ME**, Cunningham D. Medical management of advanced gastric cancer. *Cancer Treat Rev* 1998; **24**: 113-118
6 **Jiang BJ**, Wang HQ, Sun RX, Gao XF, Shen H. Clinical study on the D4 lymph nodes excision in advanced gastric cancer. *World J Gastroenterol* 2000; **6** Suppl 3: 77
7 **Deng DJ**. progress of gastric cancer etiology: N-nitrosamides 1999s. *World J Gastroenterol* 2000; **6**: 613-618
8 **Tajima Y**, Yamazaki K, Makino R, Nishino N, Aoki S, Kato M, Morohara K, Kaetsu T, Kusano M. Gastric and intestinal phenotypic marker expression in early differentiated-type tumors of the stomach: clinicopathologic significance and genetic background. *Clin Cancer Res* 2006; **12**: 6469-6479
9 **Szlosarek PW**, Dalgleish AG. Potential applications of gene therapy in the patient with cancer. *Drugs Aging* 2000; **17**: 121-132
10 **Xu CT**, Pan BR. Current status of gene therapy in gastroenterology. *World J Gastroenterol* 1998; **4**: 85-89
11 **Kirn D**, Niculescu-Duvaz I, Hallden G, Springer CJ. The emerging fields of suicide gene therapy and virotherapy. *Trends Mol Med* 2002; **8**: S68-S73
12 **Walther W**, Stein U. Therapeutic genes for cancer gene therapy. *Mol Biotechnol* 1999; **13**: 21-28
13 **Somia N**, Verma IM. Gene therapy: trials and tribulations. *Nat Rev Genet* 2000; **1**: 91-99
14 **Anklesaria P**. Gene therapy: a molecular approach to cancer treatment. *Curr Opin Mol Ther* 2000; **2**: 426-432
15 **Pandha HS**, Martin LA, Rigg AS, Ross P, Dalgleish AG. Gene therapy: recent progress in the clinical oncology arena. *Curr Opin Mol Ther* 2000; **2**: 362-375
16 **Xiao B**, Shi YQ, Zhao YQ, You H, Wang ZY, Liu XL, Yin F, Qiao TD, Fan DM. Transduction of Fas gene or Bcl-2 antisense RNA sensitizes cultured drug resistant gastric cancer cells to chemotherapeutic drugs. *World J Gastroenterol* 1998; **4**: 421-425
17 **Horio Y**, Hasegawa Y, Sekido Y, Takahashi M, Roth JA, Shimokata K. Synergistic effects of adenovirus expressing wild-type p53 on chemosensitivity of non-small cell lung cancer cells. *Cancer Gene Ther* 2000; **7**: 537-544
18 **Tatebe S**, Matsuura T, Endo K, Teramachi K, Nakamura T, Sato K, Ito H. Adenovirus-mediated transfer of wild-type p53 gene results in apoptosis or growth arrest in human cultured gastric carcinoma cells. *Int J Oncol* 1999; **15**: 229-235
19 **Ohashi M**, Kanai F, Ueno H, Tanaka T, Tateishi K, Kawakami T, Koike Y, Ikenoue T, Shiratori Y, Hamada H, Omata M. Adenovirus mediated p53 tumour suppressor gene therapy for human gastric cancer cells in vitro and in vivo. *Gut* 1999; **44**: 366-371
20 **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 Huaren Xiaohua Zazhi* 2001; **9**: 39-42
21 **Jeong YW**, Kim KS, Oh JY, Park JC, Baek WK, Suh SI, Suh MH, Lee JC, Cho JW. Exogenous wild-type p16INK4A gene induces delayed cell proliferation and promotes chemosensitivity through decreased pRB and increased E2F-1 expressions. *Int J Mol Med* 2003; **12**: 61-65
22 **Ishii H**, Zanesi N, Vecchione A, Trapasso F, Yendamuri S, Sarti M, Baffa R, During MJ, Huebner K, Fong LY, Croce CM. Regression of upper gastric cancer in mice by FHIT gene delivery. *FASEB J* 2003; **17**: 1768-1770
23 **Rocco A**, Schandl L, Chen J, Wang H, Tulassay Z, McNamara D, Malfertheiner P, Ebert MP. Loss of FHIT protein expression correlates with disease progression and poor differentiation in gastric cancer. *J Cancer Res Clin Oncol* 2003; **129**: 84-88
24 **Spencer DM**. Developments in suicide genes for preclinical and clinical applications. *Curr Opin Mol Ther* 2000; **2**: 433-440
25 **Freeman SM**, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, Abraham GN. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. *Cancer Res* 1993; **53**: 5274-5283
26 **Liu HF**, Liu WW, Fang DC, Men RP. Expression and significance of proapoptotic gene Bax in gastric carcinoma. *World J Gastroenterol* 1999; **5**: 15-17
27 **Fu YG**, Qu YJ, Wu KC, Zhai HH, Liu ZG, Fan DM. Apoptosis-inducing effect of recombinant Caspase-3 expressed by constructed eukaryotic vector on gastric cancer cell line SGC7901. *World J Gastroenterol* 2003; **9**: 1935-1939
28 **Yamabe K**, Shimizu S, Ito T, Yoshioka Y, Nomura M, Narita M, Saito I, Kanegae Y, Matsuda H. Cancer gene therapy using a pro-apoptotic gene, caspase-3. *Gene Ther* 1999; **6**: 1952-1959
29 **Wei SJ**, Chao Y, Hung YM, Lin WC, Yang DM, Shih YL, Ch'ang LY, Whang-Peng J, Yang WK. S- and G2-phase cell cycle arrests and apoptosis induced by ganciclovir in murine melanoma cells transduced with herpes simplex virus thymidine kinase. *Exp Cell Res* 1998; **241**: 66-75
30 **Kwon GY**, Jeong J, Woo JK, Choi HY, Lee MJ, Ko JK, Shim YH, Kim CW. Co-expression of bfl-1 enhances host response in the herpes simplex virus-thymidine kinase/ganciclovir gene therapy system. *Biochem Biophys Res Commun* 2003; **303**: 756-763
31 **Ueda K**, Iwahashi M, Nakamori M, Nakamura M, Matsuura I, Yamaue H, Tanimura H. Carcinoembryonic antigen-specific suicide gene therapy of cytosine deaminase/5-fluorocytosine enhanced by the cre/loxP system in the orthotopic gastric carcinoma model. *Cancer Res* 2001; **61**: 6158-6162
32 **Zheng SY**, Li DC, Zhang ZD, Zhao J, Ge JF. Adenovirus-mediated FasL gene transfer into human gastric carcinoma. *World J Gastroenterol* 2005; **11**: 3446-3450
33 **Atienza C Jr**, Elliott MJ, Dong YB, Yang HL, Stilwell A, Liu TJ, McMasters KM. Adenovirus-mediated E2F-1 gene transfer induces an apoptotic response in human gastric carcinoma cells that is enhanced by cyclin dependent kinase inhibitors. *Int J Mol Med* 2000; **6**: 55-63
34 **Tsunemitsu Y**, Kagawa S, Tokunaga N, Otani S, Umeoka T, Roth JA, Fang B, Tanaka N, Fujiwara T. Molecular therapy for peritoneal dissemination of xenotransplanted human MKN-45 gastric cancer cells with adenovirus mediated Bax gene transfer. *Gut* 2004; **53**: 554-560
35 **Takimoto R**, Kato J, Terui T, Takada K, Kuroiwa G, Wu J, Ohnuma H, Takahari D, Kobune M, Sato Y, Takayama T, Matsunaga T, Niitsu Y. Augmentation of antitumor effects of p53 gene therapy by combination with HDAC inhibitor. *Cancer Biol Ther* 2005; **4**: 421-428
36 **Kunisaki R**, Ikawa S, Maeda T, Nakazaki Y, Kurita R, Harata M, Shutoh Y, Bai YS, Soda Y, Tanabe T, Dohi T, Kato R, Ikawa Y, Asano S, Tani K. p51/p63, a novel p53 homologue, potentiates p53 activity and is a human cancer gene therapy candidate. *J Gene Med* 2006; **8**: 1121-1130
37 **Nishimura S**, Adachi M, Ishida T, Matsunaga T, Uchida H, Hamada H, Imai K. Adenovirus-mediated transfection of caspase-8 augments anoikis and inhibits peritoneal dissemination of human gastric carcinoma cells. *Cancer Res* 2001; **61**: 7009-7014
38 **Tian X**, Wu J, Meng L. Preparation of monoclonal antibodies to human vascular endothelial growth factor(VEGF121) and identification of its expression on gastric carcinoma cell line

MGC803. *Zhonghua Zhongliu Zazhi* 1999; **21**: 93-95
39 **Giatromanolaki A**, Koukourakis MI, Stathopoulos GP, Kapsoritakis A, Paspatis G, Kakolyris S, Sivridis E, Georgoulias V, Harris AL, Gatter KC. Angiogenic interactions of vascular endothelial growth factor, of thymidine phosphorylase, and of p53 protein expression in locally advanced gastric cancer. *Oncol Res* 2000; **12**: 33-41
40 **Stoeltzing O**, McCarty MF, Wey JS, Fan F, Liu W, Belcheva A, Bucana CD, Semenza GL, Ellis LM. Role of hypoxia-inducible factor 1alpha in gastric cancer cell growth, angiogenesis, and vessel maturation. *J Natl Cancer Inst* 2004; **96**: 946-956
41 **Tandle A**, Blazer DG 3rd, Libutti SK. Antiangiogenic gene therapy of cancer: recent developments. *J Transl Med* 2004; **2**: 22
42 **Meng F**, Ding J, Liu N, Zhang J, Shao X, Shen H, Xue Y, Xie H, Fan D. Inhibition of gastric cancer angiogenesis by vector-based RNA interference for Raf-1. *Cancer Biol Ther* 2005; **4**: 113-117
43 **Xue Y**, Bi F, Zhang X, Pan Y, Liu N, Zheng Y, Fan D. Inhibition of endothelial cell proliferation by targeting Rac1 GTPase with small interference RNA in tumor cells. *Biochem Biophys Res Commun* 2004; **320**: 1309-1315
44 **Heideman DA**, van Beusechem VW, Bloemena E, Snijders PJ, Craanen ME, Offerhaus GJ, Derksen PW, de Bruin M, Witlox MA, Molenaar B, Meijer CJ, Gerritsen WR. Suppression of tumor growth, invasion and angiogenesis of human gastric cancer by adenovirus-mediated expression of NK4. *J Gene Med* 2004; **6**: 317-327
45 **Ueda K**, Iwahashi M, Matsuura I, Nakamori M, Nakamura M, Ojima T, Naka T, Ishida K, Matsumoto K, Nakamura T, Yamaue H. Adenoviral-mediated gene transduction of the hepatocyte growth factor (HGF) antagonist, NK4, suppresses peritoneal metastases of gastric cancer in nude mice. *Eur J Cancer* 2004; **40**: 2135-2142
46 **Sako A**, Kitayama J, Koyama H, Ueno H, Uchida H, Hamada H, Nagawa H. Transduction of soluble Flt-1 gene to peritoneal mesothelial cells can effectively suppress peritoneal metastasis of gastric cancer. *Cancer Res* 2004; **64**: 3624-3628
47 **Miller N**, Whelan J. Progress in transcriptionally targeted and regulatable vectors for genetic therapy. *Hum Gene Ther* 1997; **8**: 803-815
48 **Mizuguchi H**, Hayakawa T. Targeted adenovirus vectors. *Hum Gene Ther* 2004; **15**: 1034-1044
49 **Fechner H**, Haack A, Wang H, Wang X, Eizema K, Pauschinger M, Schoemaker R, Veghel R, Houtsmuller A, Schultheiss HP, Lamers J, Poller W. Expression of coxsackie adenovirus receptor and alphav-integrin does not correlate with adenovector targeting in vivo indicating anatomical vector barriers. *Gene Ther* 1999; **6**: 1520-1535
50 **Okegawa T**, Li Y, Pong RC, Bergelson JM, Zhou J, Hsieh JT. The dual impact of coxsackie and adenovirus receptor expression on human prostate cancer gene therapy. *Cancer Res* 2000; **60**: 5031-5036
51 **Li D**, Duan L, Freimuth P, O'Malley BW Jr. Variability of adenovirus receptor density influences gene transfer efficiency and therapeutic response in head and neck cancer. *Clin Cancer Res* 1999; **5**: 4175-4181
52 **Bischoff JR**, Kirn DH, Williams A, Heise C, Horn S, Muna M, Ng L, Nye JA, Sampson-Johannes A, Fattaey A, McCormick F. An adenovirus mutant that replicates selectively in p53-deficient human tumor cells. *Science* 1996; **274**: 373-376
53 **Gossen M**, Bujard H. Tight control of gene expression in mammalian cells by tetracycline-responsive promoters. *Proc Natl Acad Sci USA* 1992; **89**: 5547-5551
54 **Aberle S**, Schug N, Mathlouthi R, Seitz G, Kupper JH, Schroder K, Blin N. Promoter selection for the cytosine deaminase suicide gene constructs in gastric cancer. *Eur J Gastroenterol Hepatol* 2004; **16**: 63-67
55 **Nakaya H**, Ishizu A, Ikeda H, Tahara M, Shindo J, Itoh R, Takahashi T, Asaka M, Ishikura H, Yoshiki T. In vitro model of suicide gene therapy for alpha-fetoprotein-producing gastric cancer. *Anticancer Res* 2003; **23**: 3795-3800
56 **Ono HA**, Davydova JG, Adachi Y, Takayama K, Barker SD, Reynolds PN, Krasnykh VN, Kunisaki C, Shimada H, Curiel DT, Yamamoto M. Promoter-controlled infectivity-enhanced conditionally replicative adenoviral vectors for the treatment of gastric cancer. *J Gastroenterol* 2005; **40**: 31-42
57 **Heideman DA**, Snijders PJ, Craanen ME, Bloemena E, Meijer CJ, Meuwissen SG, van Beusechem VW, Pinedo HM, Curiel DT, Haisma HJ, Gerritsen WR. Selective gene delivery toward gastric and esophageal adenocarcinoma cells via EpCAM-targeted adenoviral vectors. *Cancer Gene Ther* 2001; **8**: 342-351
58 **Heideman DA**, van Beusechem VW, Offerhaus GJ, Wickham TJ, Roelvink PW, Craanen ME, Pinedo HM, Meijer CJ, Gerritsen WR. Selective gene transfer into primary human gastric tumors using epithelial cell adhesion molecule-targeted adenoviral vectors with ablated native tropism. *Hum Gene Ther* 2002; **13**: 1677-1685
59 **Tanaka T**, Kanai F, Okabe S, Yoshida Y, Wakimoto H, Hamada H, Shiratori Y, Lan K, Ishitobi M, Omata M. Adenovirus-mediated prodrug gene therapy for carcinoembryonic antigen-producing human gastric carcinoma cells in vitro. *Cancer Res* 1996; **56**: 1341-1345
60 **Terazaki Y**, Yano S, Yuge K, Nagano S, Fukunaga M, Guo ZS, Komiya S, Shirouzu K, Kosai K. An optimal therapeutic expression level is crucial for suicide gene therapy for hepatic metastatic cancer in mice. *Hepatology* 2003; **37**: 155-163
61 **Richards CA**, Austin EA, Huber BE. Transcriptional regulatory sequences of carcinoembryonic antigen: identification and use with cytosine deaminase for tumor-specific gene therapy. *Hum Gene Ther* 1995; **6**: 881-893
62 **Thompson J**, Epting T, Schwarzkopf G, Singhofen A, Eades-Perner AM, van Der Putten H, Zimmermann W. A transgenic mouse line that develops early-onset invasive gastric carcinoma provides a model for carcinoembryonic antigen-targeted tumor therapy. *Int J Cancer* 2000; **86**: 863-869
63 **Tanaka T**, Huang J, Hirai S, Kuroki M, Kuroki M, Watanabe N, Tomihara K, Kato K, Hamada H. Carcinoembryonic antigen-targeted selective gene therapy for gastric cancer through FZ33 fiber-modified adenovirus vectors. *Clin Cancer Res* 2006; **12**: 3803-3813
64 **Ueda K**, Iwahashi M, Matsuura I, Nakamori M, Nakamura M, Ojima T, Naka T, Ishida K, Matsumoto K, Nakamura T, Yamaue H. Adenoviral-mediated gene transduction of the hepatocyte growth factor (HGF) antagonist, NK4, suppresses peritoneal metastases of gastric cancer in nude mice. *Eur J Cancer* 2004; **40**: 2135-2142
65 **Romano M**, Ricci V, Zarrilli R. Mechanisms of disease: Helicobacter pylori-related gastric carcinogenesis--implications for chemoprevention. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 622-632
66 **Sugiyama T**. Development of gastric cancer associated with Helicobacter pylori infection. *Cancer Chemother Pharmacol* 2004; 54 Suppl 1: S12-S20
67 **Bretagne JF**. Could Helicobacter pylori treatment reduce stomach cancer risk? *Gastroenterol Clin Biol* 2003; **27**: 440-452

S- Editor Liu Y **L- Editor** Alpini GD **E- Editor** Lu W