Gene therapy for gastric diseases

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Abstract: Gene therapy for gastric cancer and gastric ulcer is a rationalized strategy since various genes correlate with these diseases. Since gene expressions in non-target tissues/cells cause side effects, a selective gene delivery system targeted to the stomach and/or cancer must be developed. The route of vector transfer (direct injection, systemic, intraperitoneal, gastric serosal surface and oral administration) is an important issue which can determine efficacy and safety. Strategies for cancer gene therapy can be categorized as suicide gene therapy, growth inhibition and apoptosis induction, immunotherapy, anti-angiogenesis, and others. Combination of the target gene with other genes and/or strategies such as chemotherapy and virotherapy is promising. Candidates for treatment of gastric ulcer are vascular endothelial growth factor, angiopoietin-1, serum response factor, and cationic host defense peptide cathelicidin. In this review, we discuss stomach- and cancer-targeted gene transfer methods and summarize gene therapy trials for gastric cancer and gastric ulcer.

Keywords: Gastric cancer, carcinoma, gastric ulcer, gene delivery, stomach
INTRODUCTION

The stomach is a digestive organ that is essential for nutrient intake. An acidic environment in the stomach is required for the digestive system and a barrier function against acidic pH is necessary. Alcohol, drugs, stress, and Helicobacter pylori (H. pylori) reduce barrier function and cause gastritis and gastric ulcer. Moreover, gastric cancer is one of the most common malignant tumors in the world. It was the second most common cause of death from cancer (700,000 deaths annually) after lung cancer in 2002, according to the International Agency for Research on Cancer [1]. In 2002, the incidence of gastric cancer was estimated to be 934,000 new cases per year worldwide [1]. The mortality due to Japanese gastric cancer was over 50,000 deaths annually in 2005 according to the Center for Cancer Control and Information Services, National Cancer Center, Japan. Surgical resection of gastric cancer without metastasis is a first-line therapy and provides relatively good prognosis compared to other cancers. However, recurrence, liver metastasis and/or peritoneal dissemination in gastric cancer are serious problems with high mortality. Advanced gastric cancer does not generally respond to conventional chemotherapy or radiotherapy [2].

To treat gastric cancer and gastric ulcer, gene therapy is a promising approach because mutation, overexpression and insufficient expression of various genes correlate with these diseases. Gene delivery systems in vivo can be categorized as viral [3-5] and non-viral [6-8] approaches. Although the viral vectors have not yet proved to be a safe gene delivery method [9, 10], the transfection efficiency of viral vectors is generally much higher than that of non-viral vectors. Not only transgene expression, but also down-regulation of internal genes, is possible.
using antisense oligodeoxyribonucleotides [11] and siRNA [12, 13]. Selection of administration routes for gene therapy is an important issue, along with vector and target gene selection. In this review, we discuss stomach-targeted gene transfer methods and summarize gene therapy trials for gastric cancer and gastric ulcer.

**SELECTIVE DELIVERY OF GENES TO THE STOMACH OR GASTRIC CANCER**

Since it is thought that gene transfer to non-target organs/cells can result in severe adverse effects, selective gene transfer to target organ/cells, i.e. the stomach or cancer, is a major challenge in gene therapy, and a gene delivery system targeted to the stomach or cancer should be developed. The administration route is an important factor since it can greatly affect biodistribution of vectors. Active targeting to cancer cells has also been tried by several groups. More than half of advanced gastric cancers overexpress the carcinoembryonic antigen (CEA) protein [2, 14]. CEA is an attractive target since its expression in normal cells is generally low. Khare *et al.* developed a murine leukemia virus-based recombinant retroviral vector that displays a chimeric envelope protein containing a single-chain variable fragmented antibody to CEA [15]. Normally, wild-type ecotropic viruses do not infect human cells. In contrast, this vector infected human CEA-expressing cells. Tanaka *et al.* constructed a genetically modified adenovirus incorporating an IgG Fc-binding motif from the *Staphylococcus* protein A, Z33, within the HI loop (Adv-FZ33) [16]. Combination of Adv-FZ33 with an anti-CEA monoclonal antibody resulted in 20 times higher LacZ or EGFP gene expression than that with a control antibody in MKN-45 gastric cancer cell line. Notably, this system is easily applicable to other antibodies.
Epithelial cell adhesion molecule (EpCAM) is another target for tumor-selective gene transfer. Heideman et al. reported that EpCAM-targeted adenoviral vector using bispecific antibodies against the adenovirus fiber-knob protein and EpCAM selectively infected gastric and esophageal cancer cell lines [17].

On the other hand, a cancer-specific promoter is another tool achieving cancer-specific gene expression. A CEA promoter is commonly used for this purpose [18-20]. However, the gene expression level of tumor-specific promoters such as CEA promoter is generally low. To resolve this problem, Ueda et al. designed a Cre/loxP system under the control of a CEA promoter, and gene expression (under the control of a strong promoter) was markedly enhanced while maintaining specificity using the Cre/loxP regulation system [21]. Cyclooxygenase-2 (COX-2) promoter is also an attractive promoter since its activity is high in gastrointestinal cancer (or inflammation site) while its activity in most tissues such as the liver is low [22-24]. Yamamoto et al. demonstrated that COX-2 promoter was less active than cytomegarovirus (CMV) promoter in the liver, lung and kidney after tail vein injection of adenoviral vector, whereas activity of COX-2 promoter in subcutaneous tumors of gastric cancer cells after intratumoral injection was similar level with that of CMV promoter [25].

**ROUTES OF VECTOR TRANSFER**

Routes of vector transfer into the stomach (summarized in Fig. (1) and Table 1) can theoretically determine the efficacy and safety of vectors. When foreign genes are administered *via* the vasculature route, they are distributed to the whole body through the bloodstream, leading
to inadequate stomach-selective or disease site-selective gene delivery. Injection of vectors into the gastric artery may resolve this problem; however, there are no reports about stomach-selective gene transfer via the gastric artery. As for tumors, there are considerable numbers of reports about cancer-targeted delivery systems via the vasculature route. One such system is the PEGylated carrier system [26]. PEGylated carriers accumulate in the tumor via an enhanced permeability and retention (EPR) effect [27]. Active targeting systems utilizing tumor-specific surface receptors have also been developed [28-30]. However, no researchers have achieved stomach- or gastric cancer-selective gene transfer via the systemic circulation. Thus, it may be necessary to select other routes for stomach-targeted gene therapy.

**Direct injection to the stomach**

When plasmid DNA is injected directly into muscle, efficient transfection activity can be obtained without any carriers [31]. This method has been applied to the liver [32], heart [33], spleen [34], tumor [35], and also to the stomach [36]. However, multiple injections are necessary for gene transfer to a large population of gastric cells. Moreover, there is great concern about safety because this method requires physical force against the organs; consequently, repeated administration of plasmid DNA is limited, except for solid tumors.

**Serosal route**

The prognosis of gastric cancer patients with serosal invasion is poor [37]. The survival rate is also influenced by peritoneal dissemination [38]. Therefore, the serosal route of gene
transfer is attractive to treat serosal invasion and peritoneal dissemination of gastric cancer. Intraperitoneal injection has been widely applied as a gene transfer route [20, 39, 40]. Since intraperitoneal injection of vectors can result in non-specific gene transfer into non-target sites/cells in the peritoneal cavity, an active targeting system and/or cancer-specific promoter is required for safety.

The stomach-selective gene transfection method is expected to be a safe and effective treatment against gastric cancer localized in the stomach. We have developed an organ-selective gene transfer method, instilling naked plasmid DNA onto the mesothelial surface of target organs including the liver [41], unilateral kidney [42], spleen [43], unilateral lung [44], and stomach [45, 46] in mice. Although frequent dosing using this method is limited due to necessity of laparoscopy, application of an implantable infusion pump will prolong duration of gene expression. Gastric serosal surface instillation of plasmid DNA encoding an anti-cancer gene is thought to act primarily against the invasion of gastric cancer to the serosal side, although it may be difficult for plasmid DNA to penetrate into the mucosal side. However, prophylactic gastric serosal instillation of plasmid DNA combined with the resection of mucosal cancer might be useful to prevent invasion of the remaining cancer cells. Furthermore, we previously reported that gastric serosal surface application of the anti-cancer drug 5-fluorouracil (5-FU) resulted in comparable distribution of 5-FU between the mucosal and serosal sides in rats; consequently, 5-FU was efficiently distributed to the mucosal side from the serosal side prior to distribution to the systemic circulation [47]. Simultaneous administration of gene medicine and anti-cancer drugs onto the gastric serosal surface may thus be a promising strategy against gastric cancer.
Oral route

The oral route is the most attractive and challenging route. Non-invasive administration is possible by the oral route. Potential daily intake is also one of the merits of oral administration of vectors. Moreover, since gastric cancer and gastric ulcer are firstly generated on the mucosal side of the stomach, vectors can act against these diseases effectively. However, the epithelial barrier, low pH and digestive fluid are major obstacles for gene transfer. Shao et al. reported that the in vivo stability of a recombinant adeno-associated virus type 2 vector could be improved by gastric acid neutralization with sodium bicarbonate and protease inhibition with aprotinin [48]. Despite these changes, transduction after oral administration of this vector remained low. (Shao et al. did not find β-galactosidase-positive cells in any of the mice.) We also failed to detect transgene expression after intragastric injection of plasmid DNA in mice [46]. To overcome these obstacles, microparticles and nanoparticles are a promising approach. Chitosan-DNA microparticles could protect the encapsulated plasmid DNA from nuclease degradation [49]. In in vivo-animal studies, a blue color was observed with X-gal staining of histological stomach and small intestine sections after oral administration of chitosan-DNA microparticles. More recently, Zheng et al. reported that chitosan nanoparticles using quaternized chitosan (60% trimethylated chitosan) which were given via a gastric feeding tube exhibited green fluorescent protein expression in the mucosa of the stomach, duodenum, jejunum, ileum, and large intestine [50]. Furthermore, Bhavsar and Amiji developed a hybrid system dubbed the nanoparticles-in-microsphere oral system (NiMOS) which consists of gelatin nanoparticles.
(containing plasmid DNA) and a poly(epsilon-caprolactone) outer shell [51]. NiMOS resided in the stomach and small intestine for relatively longer than gelatin nanoparticles alone. Future application of these systems to viral vectors may improve transduction efficiency. It may be difficult for an oral delivery system alone to achieve stomach-selective gene transfer; therefore, combination with an active targeting system and/or cancer-specific promoter will be required.

Gene transfer via these routes may result in gene expression in a limited region, i.e., administration site (mucosal or serosal sides for the oral or serosal route, or the submucosal and muscular layers for direct injection). Combination of these routes can theoretically resolve this problem.

**APPROACHES TO TREAT GASTRIC CANCER**

Several approaches to treat gastric cancer have been reported. These can be categorized as suicide gene therapy, growth inhibition and apoptosis induction, immunotherapy, anti-angiogenesis, and others (Table 2). Since the efficacy of single gene transfer is generally low, combination of several strategies should be considered.

**Suicide gene therapy**

The narrow therapeutic range of anti-cancer drugs is major problem of chemotherapy; frequently, effective and toxic doses are inverted. To improve the efficacy of anti-cancer drugs, suicide gene therapy is a promising approach [52]. Suicide gene therapy is defined as a combination of a suicide gene with a less-toxic prodrug, such as herpes simplex virus thymidine
kinase (HSVtk) with ganciclovir (GCV) and Escherichia coli cytosine deaminase (CD) with 5-fluorocytosine (5-FC). If a suicide gene is selectively transferred to the tumor cells, the systemically administered prodrug can be converted to its active form in tumor cells. Moreover, suicide gene therapy is effective even with small populations of suicide gene-positive cells because the active form of the prodrug can spread from a suicide gene-positive cell to neighboring cells via gap junctions. This effect is known as the “bystander effect”, which was originally described as a phenomenon that HSVtk-positive cells sensitive to GCV were toxic to nearby tumor cells resistant to GCV [53]. Tanaka et al. tested a recombinant adenovirus vector carrying the HSVtk gene coupled to the CEA promoter for human gastric carcinoma cells in vitro [18]. The 50% growth inhibitory concentrations (IC50) of GCV were 21 and 5.8 μM for CEA-producing gastric carcinoma cell lines MKN28 and MKN45, while IC50 in a CEA-non-producing cancer cell line MKN1 was 320 μM. This system, i.e. a suicide gene under the control of a cancer-specific promoter, is also effective in vivo. Tanaka et al. continuously tested the in vivo effect of the same system, and intratumoral injection of the vector with intraperitoneal injection of GCV inhibited the growth of subcutaneous tumor xenografts of gastric cancer by 20% compared to untreated tumors [19]. Lan et al. reported that intraperitoneal injection of an adenovirus carrying the CD gene under the control of a CEA promoter with 5-FC suppressed tumor growth and prolonged survival of mice in MKN45 tumor xenograft model, suggesting this system is also effective against peritoneal dissemination [20]. In contrast, significant hepatic toxicity was noted in animals treated with an adenovirus carrying the CD gene under the control of a non-specific strong promoter (CAG promoter), suggesting that selective
Growth inhibition and apoptosis induction

Since abnormal gene expression and/or mutation of various oncogenes and tumor-suppressor genes correlate to proliferation and survival of tumor cells, gene therapy is rational. Suppression of oncogenes and/or correction of function of tumor-suppressor genes can inhibit tumor growth and induce apoptosis of tumor cells. Apoptotic signaling pathways are summarized in Fig. (2).

The functional loss of the tumor-suppressor p53 gene, the guardian of the genome, is the most common event in carcinogenesis [54]. p53 participates in several pathways of the cell cycle, including activation of genes that inhibit cell cycle progression into the S phase, promotion of DNA repair, and induction of apoptosis [55]. Point mutations of the p53 gene have been reported in more than 60% of gastric cancer cases and this leads to genetic instability and uncontrolled cell proliferation [56]. Restoration of p53 function has been shown to stabilize the malignant phenotype of neoplastic cells [57, 58]. Overexpression of wild-type p53 induces growth arrest and/or apoptosis of p53-mutated gastric cancer cells in vitro [59]. Moreover, Ohashi et al. reported that p53-specific growth inhibition after transduction with adenoviruses encoding wild-type p53 was observed in vitro in two (MKN1 and MKN7) of four gastric cancer cell lines with mutated p53, but not in a wild-type p53 cell line (MKN45) [60]. The mechanism of gastric cancer cell death was found to be apoptosis. In this report, in vivo studies showed that the growth of subcutaneous tumors of p53 mutant MKN1 cells was inhibited by direct injection of an
adenovirus, while no growth inhibition was observed in p53 wild-type MKN45 tumors. p51 (p73L/p63/p40/KET), a p53 homologue [61, 62], is another candidate of cancer gene therapy. p51 binds to p53-responsive elements to up-regulate some p53 target genes and has been suggested to share partially overlapping functions with p53 [63-65]. Kunisaki et al. tested the growth-suppressive effects of adenoviral transfer of p51A in several cancer cells including gastric cancer in vitro, and observed a significant anti-tumor effect on day 6 in all kinds of tumors analyzed irrespective of the expression level of endogenous p51 [66].

Cancer cells expressing wild-type p53 are relatively resistant to gene transfer of p53 [67] including gastric cancer cells [60]. Activation of p53 leads to G1 arrest through induction of p21 expression, or apoptosis by activation of bax expression; however, the latter does not occur in all cells [55]. Therefore, direct induction of apoptosis by proapoptotic gene transfer is rational. Bax is a strong proapoptotic gene that causes cytochrome c release from mitochondria and subsequently activates the caspase pathway leading to apoptosis [68, 69]. It was demonstrated that adenovirus-mediated bax gene transfer could effectively suppress tumor growth in both p53-sensitive and p53-resistant human lung carcinoma cell lines [70]. As for a gastric cancer model, Tsunemitsu et al. reported that adenoviral bax treatment was more effective in suppressing both subcutaneous and peritoneally disseminated MKN-45 tumors than p53 treatment [71]. On the other hand, down-regulation of anti-apoptotic genes is also an important strategy. Overexpression of the anti-apoptotic protein bcl-2 has been associated with drug resistance in various human malignancies; the expression of bcl-2 was down-regulated in chemotherapy responders, whereas its expression was increased or unchanged in non-responders [72, 73]. The
introduction of the bcl-2 gene in vitro was followed by decreased drug sensitivity in tumor cells [74, 75]. These findings suggest that bcl-2 affects responsiveness to chemotherapy in tumor cells. Overexpression of bcl-2 was confirmed in gastric carcinoma [76]. Kim et al. reported that down-regulation of bcl-2 by antisense bcl-2 enhanced the anti-tumor effect of cisplatin (CDDP) and paclitaxel in MKN45 gastric carcinoma xenografts in vivo [77]. In contrast, silencing bcl-XL, a bcl-2 family anti-apoptotic protein, by siRNA increased spontaneous apoptosis without simultaneous drug treatment in MGC-803 gastric carcinoma cells in vitro [78].

Rho family proteins, members of the Ras superfamily of small GTPases, have been shown to regulate several signal transduction pathways, and are involved in a variety of biological processes such as cell morphology [79, 80], motility [81], proliferation [82], and apoptosis [83]. Several reports have shown that RhoA expression is up-regulated in cancers, including gastric cancer [84]. Interleukin-6 (IL-6) can promote AGS gastric carcinoma cell motility and invasiveness via activation of the c-Src/RhoA/ROCK signaling pathway; thus, high expression of RhoA in gastric cancer cells is highly correlated with aggressive lymph node metastasis, more advanced tumor stage, histologically diffuse type and poor survival [85]. Liu et al. reported that inhibition of RhoA by RhoA-specific siRNA or dominant-negative RhoA expressions reversed the malignant phenotype of gastric cancer cells in vitro [86]. Sun et al. demonstrated that a combination of RhoA and RhoC siRNA-expressing adenoviral transfer could inhibit the proliferation and invasiveness activity of gastric carcinoma SGC7901 cells [87].

The Fas ligand (FasL), a member of the tumor necrosis factor family, initiates apoptosis by binding to its surface receptor Fas (CD95) and subsequent activation of caspase cascades [88].
Gastric carcinoma SGC-7901 cells infected with adenovirus encoding FasL resulted in decreased cell growth and colony-forming activity in vitro and in vivo tumor xenografts (intratumoral injection) [89]. Pro-caspase-8 is a downstream molecule of Fas and is activated by FasL-Fas binding [90]. Activated caspase-8 can activate executioner caspases such as caspase-3 [91]; therefore, caspase-8 is attractive as a gene transfer candidate. However, overexpression of caspase-8 can result in self-oligomerization and subsequent activation in the absence of any apoptotic signal [92], which leads to non-specific cell death. Nishimura et al. designed an adenovirus encoding pro-caspase-8 for induction of anoikis, a kind of apoptosis in detached cells [93]. This system would act selectively against detached cells such as peritoneal dissemination of gastric carcinoma cells. In fact, they successfully demonstrated selective suppression of peritoneal dissemination with this system, while there were no distinct effects on cell viability or growth either of attached MKN45 cells or s.c. tumor growth in SCID mice. Gene transfer of the active form of executioner caspase-3 might more directly induce apoptosis of cancer cells. Srinivasula et al. generated constitutively active caspase-3 by switching the order of its two subunits (and combining them with a linker) [94]. This reversed-caspase-3 (rev-caspase-3) was applied to gastric cancer cells and the growth of SGC7901 cells was suppressed in a time-dependent manner [95].

Nuclear factor-kappa B (NF-κB) is a family of stimulus-induced transcription factors that plays important roles in developmental and immune response [96, 97]. The NF-κB pathway also has a role in tumor initiation, survival, and progression [98-101]. Tumor necrosis factor (TNF), a major mediator of apoptosis, also induces cell survival signals such as activation of
NF-κB [102]. Blocking NF-κB activation by inhibiting the activity of the proteasome enhanced TNF-α-induced apoptosis in gastric cancer cell line [103]. On the other hand, Smad7 is an inhibitory Smad, blocking TGF-β-induced Smad activation, terminating the signaling pathway by feedback regulation [104]. In addition, Smad7 has other TGF-β-independent functions, as well as TGF-β-dependent functions. One of the TGF-β-independent functions of Smad7 is inhibition of NF-κB activation [105, 106]. More recently, Hong et al. demonstrated that liposome-mediated transfection of Smad7 could overcome the TNF resistance in gastric cancer cells by inhibiting NF-κB activation [107].

The phosphatase and tensin homolog gene (PTEN), a tumor suppressor gene, is often mutated in various cancer cells [108]. Wild-type PTEN dephosphorylates the lipid second messenger phosphatidylinositol 3,4,5-triphosphate generated by phosphatidylinositol 3-kinase (PI3K) [109]. This function of PTEN negatively regulates PI3K/PKB/Akt-dependent cell survival, proliferation and migration [110, 111]. Although both mutation and deletion of PTEN in gastric cancer account for few cases [112], adenovirus-mediated transfer of PTEN can suppress tumor growth in in vitro gastric cancer cells and in vivo tumor xenografts [113], suggesting that overexpression of PTEN may be effective for growth inhibition of gastric cancer cells which express wild-type PTEN.

Recently, it was reported that the tumor suppressor Fhit was a repressor of β-catenin transcriptional activity [114]. Clements et al. reported that β-catenin nuclear localization occurred in approximately 33% of gastric tumors and that β-catenin mutations occur in both diffuse- and intestinal-type gastric cancers [115]. Dumon et al. inhibited forestomach tumor development by
oral gene transfer using adenoviral or adenoassociated viral vectors expressing the Fhit gene in Fhit-deficient mice [116]. The same group also found that reduced bcl-2, increased bax expression, and increased TUNEL-positive apoptotic nuclei characterized the restored epithelia of the Fhit-transduced forestomach [117]. On the other hand, Dvory-Sobol et al. constructed a recombinant adenoviral vector that carried a lethal gene (p53 up-regulated modulator of apoptosis, Puma) under the control of a β-catenin/T-cell factor (Tcf)-responsive promoter [118]. This vector inhibited cell growth in AGS gastric cancer cells that possessed an active β-catenin/Tcf pathway.

Survivin is expressed in mitosis in a cell cycle-dependent fashion [119]. It is potentially involved in both the inhibition of apoptosis and control of cell division [120, 121]. Tu et al. demonstrated that suppression of survivin expression in gastric cancer cells by stable transfection of an antisense-expressing plasmid or a dominant-negative mutant of survivin could inhibit \textit{in vivo} tumorigenesis and angiogenesis after inoculation of these cells [122].

\textbf{Immunotherapy}

In cancer patients, immune responses against cancer cells are generally insufficient. To enhance anti-cancer immunity, gene transfer of cytokine gene(s) has been investigated [123, 124]. As for gastric cancer, it was reported that the tumorigenicity of gastric carcinoma cells engineered to produce IL-2 was reduced in SCID mice reconstituted with peripheral blood cells from cancer patients, while IL-6 had no such effect [125]. On the other hand, Tanaka \textit{et al.} demonstrated that intraperitoneal IL-10 gene transfer to the peritoneal mesothelium using an adenoviral vector suppressed peritoneal dissemination of MKN45 gastric cancer cells [126]. However, IL-10 is an
immunosuppressive cytokine [127, 128]. In fact, Sakamoto et al. reported that the prognosis of patients with gastric cancer expressing IL-10 was significantly worse than those without IL-10 expression [129]. In addition, Lundin et al. showed that the peripheral blood T-cell response to H. pylori in gastric adenocarcinoma patients is characterized by decreased proliferation of CD8+ T cells and IFN-γ production by CD4+ T cells, and markedly increased production of IL-10 by cells both from the peripheral blood and gastric mucosa [130]. Therefore, further studies may be required on the usage of IL-10 prior to future clinical trials.

A DNA vaccine has the potential to induce specific, effective and persisting immunity against cancer [131]. A heterologous prime-boost strategy for effective generation of cellular immunity consists of priming the immune system by a first vector and selective boosting this immunity by a second and distinct vector [132]. Lin et al. developed a heterologous prime-boost regime using a first oral DNA vaccine (attenuated Salmonella typhimurium containing plasmid DNA) and a second adenoviral vaccine combined with a gastric cancer-specific tumor associated antigen MG7-Ag mimotope [133]. They successfully enhanced immune response against gastric cancer compared to a homologous prime-boost regime using an oral DNA vaccine or adenoviral vaccine alone.

Intercellular adhesion molecule (ICAM)-1 served as the counter-receptor for leukocyte function-associated antigen (LFA)-1 [134]. The ICAM-1/LFA-1 interaction is critical to the binding between effector cells and cancer cells, and ICAM-1 is a costimulatory molecule for immune activation [135]. Sunami et al. reported that transfection of the ICAM-1 gene inhibited lymph node metastasis of gastric cancer [136]. The same group also reported that gastric cancer
cells overexpressing ICAM-1 have a tendency to cause regression of peritoneal dissemination [137]. In addition, ICAM-2, second ligand for LFA-1, has structural and functional homology to ICAM-1 [138]. Tanaka et al. demonstrated that intratumoral injection of adenoviral ICAM-2 inhibited the growth of s.c. gastric tumors, and also that mice with peritoneal metastasis survived for a longer time after adenoviral ICAM-2 transfer than controls [139].

**Anti-angiogenesis**

Angiogenesis is required for invasive tumor growth and metastasis because avascular tumors are severely restricted in their growth potential due to the lack of a blood supply. [140]. Various factors such as vascular endothelial growth factor (VEGF) and angiopoietin induce neovascularization in tumors [141]. A soluble form of the VEGF receptor (sFlt-1) reduces the effects of VEGF by trapping VEGF with high affinity [142]. Sako et al. demonstrated that a single intraperitoneal injection of an adenoviral vector encoding sFlt-1 prevented peritoneal dissemination of gastric cancer cells [39]. Hepatocyte growth factor (HGF) antagonist NK4 inhibits not only HGF receptor c-Met-mediated tumor growth, invasion and metastasis, but also the angiogenic responses induced by basic fibroblast growth factor, VEGF, and HGF [143, 144]. Two groups reported that adenoviral-mediated transfer of NK4 could suppress the tumor growth, invasion, angiogenesis [145], and peritoneal metastasis [40] of gastric cancer.

Angiostatin and endostatin are potent angiogenesis inhibitors. Angiostatin is a fragment of plasminogen [146]. Liposome-mediated transfection of the angiostatin gene in gastric cancer cells inhibited tumorigenesis in nude mice [147]. Endostatin is a fragment of collagen XVIII
produced by hemangioendothelioma [148]. Zhang et al. constructed a gene therapy-virotherapy hybrid system CNHK300-mE, a replication-selective and transgene-expressing adenovirus carrying the mouse endostatin gene [149]. They demonstrated that the level of endostatin secreted from gastric cancer cells infected with CNHK300-mE was higher than those infected with a non-replicative adenovirus Ad-mE in vitro and in vivo. Furthermore, CNHK300-mE exhibited superior suppression of s.c. gastric cancer xenografts compared to CNHK300 and Ad-mE.

Hypoxia-inducible factor (HIF), a transcription factor, is an important upstream mediator of VEGF expression in cancer cells [150, 151]. Stoeltzing et al. demonstrated that gastric cancer cells which were stably transfected with a dominant-negative form of HIF-1α secreted less VEGF than a control [152]. In an s.c. tumor model, they also showed tumor growth and angiogenesis were suppressed. Therefore, HIF may be an attractive therapeutic target of gene therapy for gastric cancer using a dominant-negative form of HIF or HIF siRNA.

Rac1, a major member of the Rho GTPase family, is related to tumorigenesis [153], invasion and metastasis [154], and angiogenesis [81, 155]. Xue et al. showed that VEGF and HIF-1α were down-regulated in gastric cancer cells which were transfected with a Rac1 siRNA expression vector [156]. Raf-1, an upstream molecule in the mitogen-activated protein kinase (MAPK) cascade, may also be involved in tumor angiogenesis [157]. The same group also showed similar results in gastric cancer cells with a Raf-1 siRNA expression vector [158].

Other strategies

Khare et al. delivered inducible nitric oxide (NO) synthase specifically to CEA-positive
gastric cancer cells [15]. NO directly induces autocytoxicity and cytolysis of bystander cells.

Synthesis of DNA at chromosome ends by telomerase may be necessary for indefinite proliferation of human cells [159]. Over the last decade, it has become clear that most human cancers activate telomerase at some point during tumorigenesis, while this activity is largely absent in most normal tissues [160]. Telomerase activity is also commonly positive in gastric cancer [56]. Inhibition of telomerase activity in MKN-45 gastric cancer cells by antisense human telomerase stable transfection induces apoptosis and growth arrest [161]. Ye et al. also reported growth inhibition effects of human telomerase antisense oligodeoxyribonucleotides in poorly differentiated MKN45 and moderately differentiated SGC-7901 gastric cancer cells, but not in well differentiated MKN-28 gastric cancer cells [162].

Heat shock proteins (HSPs), molecular chaperones, are induced by various stresses [163]. HSP70 is involved in the folding of nascent polypeptide chains and translocation of precursor proteins across the membranes of organelles [164]. HSP70 also interacts with mutated or altered oncogene and tumor suppressor gene products, which may be associated with development and progression of tumors [165, 166]. HSP70 is overexpressed in gastric cancer, as well as in other tumors [167]. Zhao et al. demonstrated that HSP70 antisense oligodeoxyribonucleotides inhibited cell growth and induced apoptosis in gastric cancer cells [168]. On the contrary, HSPs is an activator of the innate immune system [169, 170] and can carry antigens to antigen-presenting cells [171]. Taking these functions of HSPs into consideration, a selective delivery system targeted to cancer cells will be required for down-regulation strategy of HSP70 in vivo.
Combination of several strategies

Cancer cells should be completely killed; otherwise, recurrence and/or metastasis will occur. Therefore, the synergistic effects of combining several approaches are promising.

One attractive partner is chemotherapy. It was reported that the combination therapy of intratumoral administration of plasmid DNA encoding the bax gene complexed with a cationic lipopolyamine and the anti-cancer drugs 5-FU or CDDP significantly enhanced the anti-tumor effect in a gastric cancer model [172]. Takimoto et al. reported that histone deacetylase inhibitor such as sodium butylate could enhance the efficacy of an adenoviral vector carrying wild type p53 in gastric cancer cells by p53 activation and viral receptor up-regulation, in addition to its anti-tumor activity [173]. E2F-1, E2F family of transcription factor, has the ability to induce both cell cycle progression and apoptosis [174, 175]. Overexpression of E2F-1 by adenoviral gene transfer induced apoptosis in human gastric carcinoma cells, and this effect was enhanced by cyclin-dependent kinase inhibitors [176]. Namiki et al., demonstrated that NK4-expression by intraperitoneal injection of ternary complexes (plasmid DNA with cationic lipids and non-histone chromatin proteins) suppressed the gefitinib-resistance induced by the interaction between fibroblasts and scirrhous gastric cancer, and eventually, this tailor-made combination synergistically decelerated disease progression by inhibiting proliferative, angiogenic and anti-apoptotic effects in disseminated peritoneal tumor tissues [177]. Min et al. reported that the combination of dominant-negative insulin-like growth factor I receptor expression using an adenoviral vector and chemotherapy was very effective against gastric cancer xenografts in vivo [178]. Guo et al. constructed a combination of HSV-tk with a cytokine gene (IL-2 and
granulocyte macrophage colony-stimulating factor) and showed a strong anti-tumor effect in a gastric cancer model [179]. In addition, several groups overcame drug resistance by gene delivery. Zhang et al. reported that ZNRD1 (zinc ribbon domain-containing 1 protein) antisense nucleic acid transfection sensitized drug-resistant gastric cancer cells to vincristine, increased adriamycin accumulation and inhibited cell proliferation [180]. Guo et al. demonstrated that overexpression of the tumor suppressor Runx3 gene could sensitize gastric cancer cells to chemotherapeutic drugs by down-regulating not only bcl-2, but also MDR-1 and MRP-1 [181].

Virotherapy using conditionally replicative viruses is a newly developed therapeutic option for cancer [182, 183]. This approach is easily combined with gene therapy by genetic modification of the same viruses. Zhang et al. developed a combination of oncolysis with virotherapy targeted to telomerase-positive cancers, and anti-angiogenesis by gene therapy using an endostatin gene [149].

Combination of gene therapy with radiation has also great potential to treat several cancers [184] including gastric cancer [178].

**APPROACHES TO TREAT GASTRIC ULCER**

A gastric ulcer is a deep necrotic lesion penetrating through the entire mucosal thickness, the muscularis mucosae, and often involves the muscularis propria [185]. Ulcer healing, a genetically programmed repair process, includes inflammation, cell proliferation, re-epithelialization, formation of granulation tissue, angiogenesis, interactions between various cells and the matrix and tissue remodeling, all resulting in scar formation [186]. Because VEGF
and angiopoietin-1 are involved in angiogenesis, Jones et al. studied whether local gene therapy with naked DNA encoding these genes into the ulcer base could accelerate ulcer healing through enhanced angiogenesis [187]. They successfully demonstrated accelerated gastric ulcer healing. Co-injection of both genes led to more complete structural restoration. The same group also demonstrated that gene therapy with a serum response factor accelerated experimental gastric ulcer healing and promoted re-epithelialization and muscle restoration [188]. Finally, local injection of plasmid DNA encoding the cationic host defense peptide cathelicidin promoted gastric ulcer healing by enhancing cell proliferation and angiogenesis [189].
CONCLUSION

In this review, we summarized stomach- and cancer-targeted gene transfer methods and therapeutic strategies for gastric diseases, including cancer and ulcer. Since gene expression in non-target tissues/cells can cause side effects, selective gene delivery systems targeted to the stomach and/or cancer cells must be developed. The route of vector transfer is an important issue which can determine efficacy and safety.

*H. pylori* activate NF-κB via MAPK cascades, as a consequence IL-8 release from AGS gastric cancer cells [190] and macrophages [191] occur. IL-8 is a chemoattractant, which stimulate significant infiltration of neutrophils into gastric mucosa, leading to chronic gastritis. IL-8 is also a mediator of angiogenesis [192]. *H. pylori* increased expression of mRNAs encoding not only IL-8, but also VEGF, angiogenin, urokinase-type plasminogen activator, and metalloproteinase-9 by gastric carcinoma cells, suggested that *H. pylori* infection might regulate angiogenesis and invasion of human gastric carcinoma [193]. As future prospects, suppression of these genes by antisense or siRNA may inhibit tumor progression and angiogenesis caused by *H. pylori* infection.

Tumor-associated macrophages (TAMs) have been shown to be symbiotically related to tumor cells; tumor cells recruit TAMs and provide them with survival factors, and in turn TAMs produce a variety of angiogenic factors in response to the tumor microenvironment [194]. Ishigami et al. reported that the degree of infiltration of TAMs positively correlated with depth of invasion, nodal status and clinical stage in gastric cancer patients [195]. Therefore, TAMs may be a potent target of gene therapy for gastric cancer as well as other cancer. As to targeted delivery to
macrophages, Kawakami et al. developed mannosylated cationic liposome/plasmid DNA complex and they successfully delivered foreign gene to macrophages in mice [196]. As well, a dominant negative monocyte chemoattractant-1 mutant (7ND) treatment inhibited TAMs recruitment and partially reduced angiogenesis and growth of melanoma [197], thus, this might also be effective for gastric cancer.

As for gastric cancer, investigations were performed mainly *in vitro* gastric cancer cell lines. Some investigations showed *in vivo* therapeutic effects in s.c. tumor xenografts of gastric cancer cells; however, there are strong doubts about their efficacy in primary gastric cancer. In contrast, gene therapy for peritoneal dissemination of gastric cancer is promising since good results were reported in an animal model similar to the *in vivo* situation. Prophylactic usage of gene therapy after surgical resection may improve patient prognosis. For this purpose, an oral gene delivery system or controlled release formula of gene medicine should be developed.
REFERENCES


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<table>
<thead>
<tr>
<th>Routes</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
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<tbody>
<tr>
<td>Systemic</td>
<td>Frequent dosing</td>
<td>Non-specificity</td>
</tr>
<tr>
<td></td>
<td>Vast distribution</td>
<td></td>
</tr>
<tr>
<td>Direct injection</td>
<td>Effective gene transfer</td>
<td>Physical force against organ</td>
</tr>
<tr>
<td></td>
<td>High selectivity</td>
<td>Limited region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Limited frequent dosing</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>Effective gene transfer</td>
<td>Low selectivity</td>
</tr>
<tr>
<td>Serosal surface</td>
<td>Effective gene transfer</td>
<td>Necessity of laparoscopy</td>
</tr>
<tr>
<td></td>
<td>High selectivity</td>
<td></td>
</tr>
<tr>
<td>Oral</td>
<td>Easily administration</td>
<td>Barriers (epithelium, gastric fluid)</td>
</tr>
<tr>
<td></td>
<td>Frequent dosing (daily intake)</td>
<td>Low selectivity</td>
</tr>
</tbody>
</table>

Table 1. Advantages and disadvantages of vector transfer routes for the stomach
<table>
<thead>
<tr>
<th>Categories</th>
<th>Genes (with drugs)</th>
<th>Vectors</th>
<th>In vitro/in vivo</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Suicide gene therapy</strong></td>
<td>HSVtk (GCV)</td>
<td>Adv</td>
<td>In vitro</td>
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</tr>
<tr>
<td></td>
<td>HSVtk (GCV)</td>
<td>Adv</td>
<td>Intratumoral</td>
<td>[19]</td>
</tr>
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<td></td>
<td>CD (5-FC)</td>
<td>Adv</td>
<td>i.p.</td>
<td>[20]</td>
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<td><strong>Growth inhibition and apoptosis induction</strong></td>
<td>p53</td>
<td>CL</td>
<td>In vitro</td>
<td>[59]</td>
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<tr>
<td></td>
<td>p53</td>
<td>Adv</td>
<td>Intratumoral</td>
<td>[60]</td>
</tr>
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<td></td>
<td>p53 (HDAC inhibitor)</td>
<td>Adv</td>
<td>Intratumoral</td>
<td>[173]</td>
</tr>
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<td></td>
<td>p51A</td>
<td>Adv</td>
<td>In vitro</td>
<td>[66]</td>
</tr>
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<td></td>
<td>Bax</td>
<td>Adv</td>
<td>Intratumural, i.p.</td>
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<td>Bcl-2 antisense (CDDP, PTX)</td>
<td>Bcl-XL siRNA</td>
<td>CL</td>
<td>In vitro</td>
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<td></td>
<td>RhoA siRNA or</td>
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<td>DN RhoA</td>
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<td>RhoA and RhoC siRNA</td>
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<td>In vitro</td>
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<td>FasL</td>
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<td>Pro-caspase-8</td>
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<td></td>
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<td>Adv, AAV</td>
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<td>Rac1 siRNA</td>
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<td>Other strategies</td>
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<td>Location</td>
<td>Ref</td>
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<td>Raf-1 siRNA</td>
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<td>HSP70 antisense</td>
<td>Naked</td>
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</table>

Figure legends

Fig. (1). Section of the gastric wall and scheme of vector transfer routes.

Fig. (2). Pathways for apoptosis induction and inhibition.
Fig. (1).
Fig. (2).