Adenoviral gene therapy in gastric cancer: A review

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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.

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].

Gastric cancer is a genetic disease developing from a multi-step process[6]. 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[7], 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 cured 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[8].

In gene therapy vectors are used to insert new genes into cells, or to switch off existing genes[9]. 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.\[14\]

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 p55 in vitro and in vivo.\[29,30\]. 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.\[31-33\].

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.\[34\]. 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.\[35\].

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.\[29\]. 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.\[36,37\].

Another method of killing cancer cells is to transfet 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.\[38,39\]. 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 (Table 1).\[41-43\].

**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.\[44-46\]. 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.\[46\]. 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 1 A review of recent methods used in gastric cancer adenoviral gene therapy

| Apoptosis inducers & tumor suppressors | Conclusion |
|----------------------------------------|------------|
| FasL                                   | Infection of human gastric carcinoma cells (SGC-7901) with Ad-Fasl showed increased expression of FASL, resulting in apoptosis.\[44\]. |
| HDAC inhibitor                         | Induces apoptosis in cancer cells expressing wild and pseudo-wild type p53 via activating the p53 through acetylation.\[45\]. |
| 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.\[46\]. |
| Bax                                    | In a study, Ad/Bax made marked Bax protein expression and effective apoptosis in MKN-1, MKN-7, MKN-28 gastric cell lines.\[47\]. |
| 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.\[48\]. |
| Caspase 8                              | Adv-Caspase 8 can selectively induce apoptosis in detached carcinoma cells.\[49\]. |
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).

| Table 2 A review of recent methods used in gastric cancer adenoviral gene therapy |
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| 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.[44] |
| Flt-1 | When administered intra peritoneally it markedly reduced the number of metastatic nodules larger than 1 mm in diameter on the peritoneal surface.[45] |
| 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.[46] |

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.[6,44]

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 of *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.[55-67]

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