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Gene therapy and gastrointestinal cancer: concepts and clinical facts

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Abstract *Background:* Principles of the treatment of gastrointestinal cancer with gene therapy evolved from the advent of techniques in molecular biology, from increasing insights into the molecular basis of tumorigenesis and from the need to develop more efficient treatment modalities. Any gene therapy approach has to take two major tasks into consideration: the therapeutic gene has to be delivered into the target cell population with high efficiency, specificity and safety, and has to act in a way that provides a benefit to the patient. *Discussion:* Data on 22 clinical trials on malignancies of the gastrointestinal tract are available. They utilize a variety of gene-delivery methods and target cell populations, and there is considerable variety among their strategies. Gene transfer is performed by injection of naked plasmid DNA and by use of DNA–liposome complexes and viral vectors. In some cases, the gene transfer is carried out *ex vivo* and the patients receive genetically modified cells, whereas other approaches deliver the vector to the target cell population *in vivo*. The theoretical concepts of gene therapy can be divided into three groups. One approach makes use of suicide genes comprising bacterial or viral genes that convert a nontoxic prodrug into a highly cytotoxic chemotherapeutic agent at the tumor site. This approach aims at higher therapeutic specificity and

fewer side effects than with the systemic delivery of cytotoxic agents. The second strategy makes an attempt to invoke the immune system to destroy malignant cells. Different strategies, such as immunization with genetically modified tumor cells or transfer of new genes to T cells, are considered to have clinical benefits. The major advantage of these immunotherapeutic approaches is the systemic effect both on the primary tumor and on metastases. The third strategy evolved from the insight that cancer is a genetic disease caused by activation of oncogenes or inactivation of tumor-suppressor genes. Compensation of genetic defects by the downregulation of activated oncogenes or the restoration of tumor-suppressor-gene functions may be able to revert the malignant phenotype of cancer cells. Of the 22 gene-therapy trials, 17 trials focus on immunotherapy. Only two trials make use of suicide genes and, in three trials, a functional copy of the p53 tumor-suppressor gene was reintroduced into malignant cells. Modalities for gene transfer and the strategies underlying gene therapy will be discussed in the context of gastrointestinal malignancies and the potential benefits for patients.

Key words Cancer · Gastrointestinal tract · Gene therapy

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Introduction

Why use gene therapy in gastrointestinal cancer? Despite improved diagnostic methods and therapeutic regimens, cancer of the gastrointestinal tract has a poor prognosis in the majority of cases. There is an increasing demand for novel diagnostic and therapeutic approaches. From new insights into the molecular basis of tumorigenesis and from the advent of recombinant DNA technology, the opportunity to treat cancer by using genetic information has emerged.

We will discuss current concepts of gene therapy for gastrointestinal cancer and the results of the first clinical trials. Since this new field has emerged rapidly and is marked by a high degree of diversity, we will not make an attempt to provide a complete enumeration of all protocols. Rather, we will focus on representative concepts and provide an overview of clinical trials published by the Office of Recombinant DNA Activities (www.nih.gov/od/orda/protocol.htm) and the clinical trials database (www.wiley.co.uk/genetherapy).

Any gene therapy approach has to deliver genetic information to the target cell population, which is one of the major technical obstacles in gene therapy. In the first part of the text, we will discuss both the DNA-transfer techniques used in ongoing trials and new promising techniques currently under investigation.

The expression of the transferred gene has to provide benefits to the patient. Several strategies, including local production of cytotoxic drugs by bacterial or viral genes, the enhancement of the host's immune response to a tumor and the re-expression of knocked-out tumor-suppressor genes in tumors, are under evaluation. In the second part of our review, these strategies will be discussed in the context of gastrointestinal malignancies and the potential benefits for patients.

Practical ways to deliver genes into cells

Injection of naked DNA

Several methods have been developed to deliver foreign genetic information into cells, but not every technique known from cell cultures was proven to be useful in gene-therapy trials. The most simple method of gene transfer is use of naked plasmid DNA without any physical, chemical or biological agents. This approach is effective at least in some tissues, such as muscle and some tumors [1].

DNA transfer by liposomes

In many studies, the plasmid DNA is complexed with cationic liposomes, which enhance uptake of DNA by

the cells and increase gene-transfer efficiency [2, 3, 4, 5]. The common disadvantage of both methods is that in vivo delivered plasmids are only maintained for a limited time within the cells, and only a minor subpopulation (if any) of the cells stably integrates the DNA [6, 7].

Viruses as natural gene-transfer vehicles

Viruses have evolved an optimized system for delivery of genetic information and expression of genes in their host cells. The high gene-transfer efficiency has led to the development of genetically engineered viral vector systems for use in gene therapy.

Retroviral vectors

Retroviral vectors are one of the most common gene-delivery systems used in human gene-therapy trials [6]. They transfer genetic information as a single-stranded RNA genome. After infection of the host cell, the RNA is reverse transcribed into DNA and is integrated stably and with high efficiency into the genome. The vectors currently in use are mainly derived from Moloney murine leukemia virus (MMLV). They are replication defective and harbor many genetic alterations that prevent recombination and formation of replication-proficient viruses, thus creating a safety issue [8]. More than a thousand patients have received retroviral vectors in clinical trials; no cases of adverse effects of the vector were observed [9]. For treatment of gastrointestinal malignancies, retroviral vectors have been utilized in five immunotherapy trials [10, 11, 12, 13]. Retroviruses, however, possess a number of disadvantages, including an inability to infect nondividing cells and an ability to harbor oncogenicity and insertional mutagenesis due to random chromosomal integration. Recombinant nucleic-acid sequences that are randomly inserted into the human genome may transform a benign cell into a tumorigenic one by activation of a proto-oncogene or by disruption of a tumor-suppressor gene [14]. MMLV-derived vectors are lysed by human complement and are, therefore, not suitable for in vivo gene transfer except in immunoprivileged organs like the brain [15]. These disadvantages have drawn increasing attention to the development of vectors based on other viruses.

Adenoviral vectors

Adenoviruses contain a linear, double-stranded genome approximately 36 kb in size. They can infect a wide variety of cell types and can transfer genes into nondividing cells [16, 17]. They are reasonably stable in vivo, and adenoviral vectors can be used to infect cells in situ [16, 17]. Most adenovirus vectors are E1 (early protein)-dele-

tion mutants and, therefore, are not replication competent [16, 18]. Novel vectors carry additional deletions within the E3 region to increase the “space” available for the introduction of therapeutic genes [16]. The vectors can be prepared at high titers, and they can direct high levels of foreign-gene expression. The extrachromosomal persistence of the adenovirus genome alleviates the risk of insertional mutagenesis but also limits the time span of foreign-gene expression to several weeks [17]. Another limitation is that most adenovirus vectors are immunogenic, and an immune response is generated against transduced cells [17, 19]. As transduced cells are destroyed by the immune response, there may be a rapid decline in the levels of the gene product. Repeated gene transfer with an adenovirus vector is generally unsuccessful, as the secondary immune response either prevents transduction or rapidly eliminates cells containing the vector [20]. The ongoing progress in vector development has led to the creation of adenoviral vectors that harbor increased modifications within the viral genome in order to reduce immunogenicity and to improve expression of the therapeutic gene [21, 22, 23, 24]. The most deleted vectors, so-called gutless vectors, contain only viral sequences needed in cis for its replication and packaging [25, 26].

Pox viruses

Two cancer-therapy trials made use of new types of gene-transfer vectors belonging to the family of pox viruses. Pox viruses are the largest and most complex viruses known. They are oval or “brick-shaped” particles with a size of 200–400 nm and are coated by two membranes. They contain a linear, double-stranded genome that encodes more than 200 genes. Pox viruses do not integrate in the genome but replicate in the cell’s cytoplasm [27]. They seem to be suitable vectors in gene therapy because of the large size of the genome and their genetic stability [28]. Currently, vectors derived from vaccinia virus and from canarypox virus are used in clinical trials [29, 30]. Humans have long been exposed to canarypox virus, but there is no known disease associated with this virus [31]. Since vaccinia virus elicits both humoral and cell-mediated immune responses [28], copresentation of recombinant proteins with vaccinia antigens may enhance immunogenicity to the desired protein; this renders vaccinia virus an interesting vector for immunotherapeutic approaches.

Viruses under investigation for gene transfer

Various other vectors are still under evaluation for their potential use in gene therapy. The human parvovirus adeno-associated virus (AAV) type 2, has a number of features that make it an attractive candidate for gene de-

livery. There is no human disease known to be associated with AAV infection [32]. Wild-type AAV has the potential to integrate site-specific into a defined region of chromosome 19 [33]. The loss of the viral rep gene in vectors derived from AAV abolishes the site specificity; therefore, AAV vectors integrate randomly throughout the genome [34]. The stable chromosomal integration permits a long-lasting expression of the therapeutic gene [32]. AAV vectors can harbor a therapeutic gene with a maximum size of approximately 4 kb, which limits its use [33, 34]. AAV vectors do not express viral genes, nor do they generate an immune response [33]. The somewhat complicated life cycle of the virus has precluded the use of the virus as a safe and effective gene-transfer vehicle until now [32, 35]. Vectors derived from other viruses, including herpes simplex virus type 1 and lentiviruses, are also under investigation [36, 37, 38, 39].

Therapeutic strategies

Suicide-gene therapy

Enzymes like thymidine kinase of herpes simplex virus (HSV-TK) or cytosine deaminase (CD) of *Escherichia coli* can convert prodrugs into cytotoxic metabolites. The presence of genes for these so-called suicide genes can thereby be utilized to selectively kill those cells expressing the gene.

E. coli CD gene

CD is an enzyme which is expressed in bacteria and fungi but not in mammalian cells. Transfer and expression of this gene in mammalian cells does not affect tumor growth, nor does the gene exhibit any cytotoxic effect. The bacterial enzyme CD catalyzes the conversion of the antimycotic prodrug 5-fluorocytosine into the chemotherapeutic agent 5-fluorouracil (5-FU) and thus provides a useful system for selective killing of genetically modified tumor cells in vivo [40, 41]. CD-expressing cells are able to kill nonexpressing cells by a bystander effect whenever gap junctions are formed between the cells [42, 43]. The CD gene is currently used in two clinical trials evaluating the immune response against adenovirus vectors [13] and one phase-I clinical study of the treatment of metastatic colon carcinoma of the liver. In this study, the CD gene is delivered by intratumoral injection of a replication-deficient adenovirus vector. Simultaneously, the prodrug 5-FU was administered orally [44].

HSV-TK gene

The second suicide gene, the herpes-simplex-virus encoded thymidine kinase (tk), converts the prodrug ganci-

clovir into ganciclovir monophosphate. Further phosphorylation by cellular kinases generates the cytotoxic compound ganciclovir triphosphate. This strategy is well known and established for the therapy of herpes-virus infections by ganciclovir. Several experimental models have demonstrated the effectiveness of this system in animal tumors [45, 46, 47, 48]. Although not all tumor cells carried the tk gene, complete eradication of the tumor was achieved, which was explained by the action of a bystander effect [46, 47]. The tk-ganciclovir system was first evaluated to treat brain tumors, and promising results [49] advocated the continuance of clinical trials. For the treatment of glioblastoma multiforme, the tk approach has been evaluated in a phase-II study [50] and has now entered a phase-III study. A phase-I study has been initiated for the treatment of liver metastases [51].

A successful therapy requires selective expression of a suicide gene in tumor cells and subsequent administration of a prodrug to eliminate tumor cells [41, 45, 46, 52]. Tumor restricted-gene expression can be achieved (in principle) in several ways. Gene transfer can either be limited to the site of the tumor by a local application of the genetic vector or by using a vector system that is only capable of transferring the genetic information to tumor cells. A different approach is to transfer the therapeutic gene to every cell of the patient but to couple expression of the suicide gene to the transformed state of the tumor cells by the use of tumor-specific promoters [53]. Whereas selective gene transfer was successfully used to treat brain tumors by implanting retroviral producer cells into the patient's brain [49], one clinical trial made use of the tumor specificity of the carcinoembryonic antigen promoter to drive tk expression in tumor cells in order to treat liver metastases [13].

Immunotherapy

The idea behind immunotherapy of cancer is that transformed cells express antigens that, in principle, can be recognized by the immune system and can be targeted for specific elimination of tumor cells [54, 55]. Potential tumor antigens are, for example, mutated oncogenes or tumor-suppressor genes that were causative for the tumor (for example the ETV6-AML1 fusion protein in lymphoblastic leukemia [56]) or proteins that are normally rarely expressed in differentiated cells but are expressed in the malignant tissue like expression of the melanoma antigen-encoding genes (MAGE) in melanoma cells [57] or carcinoembryonic antigen (CEA) [58]. The fact that a tumor has developed indicates that the aberrant cells have failed to induce an immune recognition.

The majority of gastrointestinal tumors expresses CEA, a protein normally expressed mainly in fetal gut and (in small amounts) normal colonic mucosa [59, 60]. This tumor-associated antigen is the target in four immu-

notherapy trials for the treatment of gastrointestinal cancer [29, 30, 61]. Expression of the CEA protein in cells that harbor the full repertoire of cellular functions necessary for peptide processing, major histocompatibility complex (MHC) presentation and immune stimulation can elicit an immune response against tumor cells expressing CEA and may lead to destruction of tumor cells after antigen recognition [58].

There has been a long history of clinical trials using various tumor preparations or extracts to immunize cancer patients and, despite all the accompanying problems, some patients had clinical evidence of anti-tumor effects [62, 63, 64]. Vaccination with genes expressing a defined antigen has been proven to be effective in animal models and is now being evaluated in humans.

Conry and coworkers injected plasmid DNA intramuscularly in patients with metastatic colorectal cancer [61]. As outlined before, this plasmid enters muscle cells and directs the expression of the encoded gene in these cells. Because peptide fragments of the protein encoded by the plasmid will be presented by MHC molecules and may elicit an immune response, this strategy was named polynucleotide vaccination. In the approach of Conry, a plasmid encoding human carcinoembryonic antigen is used for the anticancer treatment. In this experimental stage, they added a second plasmid encoding a hepatitis B surface antigen as a positive control for the immunization protocol [61].

Other trials are using vectors derived from the pox virus family. In two trials, a vaccinia virus was genetically engineered to express CEA [29, 30]. The induction of an immune response directed against CEA is believed to be enhanced by co-expression of vaccinia virus proteins, which are known to be immunogenic [65]. Kaufman and coworkers report that vector application induced a mild local and systemic immune reaction, but no significant complications were associated with the recombinant vaccine. In particular, neither autoimmune colitis nor leukopenia caused by homology between CEA and leukocyte antigens occurred. Most patients demonstrated tumor progression [29]. This group has now initiated a clinical trial with a canarypox-virus-derived vector expressing CEA in combination with human leukocyte antigen (HLA) B7.1 [13].

Tumor cells often lack expression of HLA antigens, which are co-stimulatory cell-surface molecules essential for the activation of T lymphocytes by MHC-presented peptide antigens. This lack can cause an immune escape of the tumor cells. The concept of reintroduction of co-stimulatory surface molecules assumes that immune recognition of tumor cells will be restored and that cells of the immune system, in particular cytotoxic T lymphocytes, will be primed to recognize and eliminate tumor cells [66, 67].

The transfer of HLA-B7 was previously adapted to the treatment of malignant melanoma without any sign

of toxicity. One of the patients showed a partial remission, and the generation of cytotoxic T lymphocytes specific for autologous tumor cells has been proved [68]. Current trials in gastrointestinal cancers have extended this concept by combination of the genes encoding HLA-B7 and β -2 microglobulin [2, 3, 69].

The proteins encoded by these two genes normally associate during their processing within the cell and are transported as a heterodimer to the cell surface. This co-expression ensures formation of the complete histocompatibility molecule. This is important, because not all tumor cells express β -2 microglobulin.

The natural stimulus for T cell activation is the recognition of an antigenic peptide bound to MHC class-II molecules. As a result of insights into the mechanisms of signal transduction of the T cell receptor, it was possible to develop strategies to circumvent the need for this stimulus by the transfer of chimeric genes to T cells. In this approach, the ζ component of the T cell receptor is genetically manipulated and equipped with an extracellular recognition domain. Introduction of this chimeric gene, consisting of the ζ chain of the T cell receptor and a single-chain antibody domain, into T lymphocytes results in T cells with a predetermined recognition specificity for particular tumor cells. The MHC restriction of target cell recognition can be avoided, and tumor cells recognized by the single chain antibody domain can be recognized as targets for T cells. Currently, clinical trials make use of this strategy, utilizing the variable domain of the CC49 antibody directed against tumor-associated antigen (TAG) 72 as the recognition domain of the chimeric gene [13, 70]. One of the trials also transfers an interferon- α gene in order to enhance TAG-72 expression [13]. In both trials, T lymphocytes are infected *ex vivo* with a retroviral vector encoding the therapeutic gene(s) and reinfused into the patient.

A promising approach in gene therapy of cancer is the local expression of immunostimulatory cytokines within the tumor. The goal of this strategy is the direction of lymphocytes to the tumor and the activation of lymphocytes to elicit an antitumoral response [71]. There have been reports on tumor suppression in mice using tumor cells transfected with various cytokine genes. The most prominent antitumoral effects have been shown for interleukin (IL) 2, IL-4, IL-7, tumor necrosis factor α and granulocyte/macrophage colony-stimulating factor (GM-CSF) [72, 73].

Three trials utilize the immunostimulatory action of IL-2. The group of Gilly injected an adenoviral vector directly into colon carcinomas [74]. Rosenberg and co-workers made attempts to immunize patients with autologous tumor cells that were *ex vivo* transduced with a retroviral vector containing the IL-2 gene [11]. In contrast, the simultaneous presence of IL-2 and tumor antigens in a cancer vaccine was achieved by a mixture of two cell types in the trial initiated by Sobol. Irradiated

autologous tumor cells served as sources of tumor antigens. IL-2 was supplied by fibroblasts expressing IL-2 from a retroviral vector [12]. Other clinical trials studied the benefit of tumor necrosis factor [10] or of the combined expression of IL-2 and IL-7 [75]. In addition, a lethally irradiated GM-CSF-transfected allogenic pancreatic cell line is being used in a clinical trial for the treatment of patients with adenocarcinoma of the head of the pancreas [76].

Expression of wild-type tumor-suppressor genes

The generally accepted model of tumorigenesis assumes that the disease is caused by accumulation of genetic alterations. Fearon and Vogelstein published a genetic model for colorectal tumorigenesis in 1990 [77]. At least two (often more) steps are required, including activation of proto-oncogenes and inactivation of tumor-suppressor genes to facilitate the progression from normal cells to benign and eventually malignant tumors.

If the functional failure of a tumor-suppressor gene is a crucial event in the development of a specific tumor, reintroduction of an intact complementary DNA copy of the defective gene can either stop the uncontrolled growth of the cells or induce apoptosis. Numerous *in vitro* and *in vivo* experimental approaches to test this hypothesis support the view that either proper cell-cycle regulation can be restored or apoptosis can be induced by the reintroduction of certain gene functions. For example, it has been shown that introduction of wild-type retinoblastoma tumor-suppressor gene (Rb) into a Rb-deficient osteosarcoma cell line reverses morphologic properties and aberrant growth [78]. In addition, restoration of wild-type p53 expression has been demonstrated to cause the malignant potential of some tumor cell lines to revert to a benign phenotype [79, 80, 81, 82, 83]. However, the p53 tumor-suppressor gene is implicated in apoptotic pathways, and p53 expression can induce apoptosis [84, 85]. Currently, three clinical studies have started with the reintroduction of an intact p53 gene into primary and metastatic liver tumors, hepatocellular carcinomas and post-hepatitis liver cancer [13, 74, 86].

Downregulation of oncogene expression

Expression of activated protooncogenes is another common cause of transformation of cells. Downregulation of oncogene expression can abrogate malignant transformation and the malignant phenotype of the cells may, thus, be reverted. Transformation caused by the overexpression of HER-2/neu is the target in a gene-therapy study (generally designed for the treatment of advanced cancer) that utilizes the expression of adenovirus E1A gene [87]. This protein downregulates HER-2/neu expression

on a transcriptional level and thereby reverts the transformed phenotype [88]. Studies concerning downregulation of oncogenes by antisense oligonucleotides or blocking of the action of oncogenic proteins by single-chain antibodies have been initiated for various types of cancer (but not for gastrointestinal malignancies) [13].

Future perspectives

Driven by the need for improved therapeutic approaches, gene therapy strategies evolved from technologies used in molecular biology and the resulting insights in the normal and pathological cellular pathways. The current gene-therapy approaches have reached a basic level. The vast majority are phase-I/II clinical trials, which are intended to determine safety of administration of the therapeutic agent. Most of the trials are still in progress. Although positive treatment results are rare, gene therapy appears to be a promising approach for future treatment of gastrointestinal malignancies, since adverse effects are rare and some patients had at least transient improvements.

Progress in targeted gene delivery and conditional gene expression may increase efficiency and reduce side effects. So far, no clinical gene-therapy trial either facilitates safe, targeted and efficient transfer of genetic information into the target cell or enables regulated expression of the therapeutic gene. Notwithstanding these limitations, the clinical protocols have shown that, for some patients, gene therapy provides an appropriate risk-to-benefit ratio. Major landmarks in future vector development will include targeted *in vivo* gene transfer and the expression of the therapeutic gene in a regulated fashion.

Efforts are underway to achieve either targeted gene transfer into tumor cells or to ensure gene expression restricted to tumor cells. Several of the clinical trials utilizing *in vivo* gene therapy performed an intratumoral injection of their vector systems, an approach which provides only limited specificity of gene transfer. More sophisticated approaches that make use of specific receptors present on the target cell populations are under development. One example is a nonviral gene-transfer system consisting of a basic fibroblast growth factor (FGF2) conjugated to polylysine as a DNA-binding moiety. FGF2 mediates binding to its cognate receptor with high affinity and the subsequent entry into the cell occurs by receptor-mediated endocytosis. It has been shown that, at least *in vitro*, therapeutically relevant suicide genes could be delivered [89]. Related approaches utilized fusion proteins of the yeast transcription factor Gal4 DNA-binding domain as a plasmid-binding moiety and a single-chain antibody fragment directed against the tumor-associated ErbB2 receptor to achieve tumor targeting. This model is further enhanced by the diphtheria-

toxin translocation domain to facilitate lysosomal escape [90]. These examples and similar approaches [91, 92] demonstrate that the combination of DNA-docking systems and any targeting domain may potentially enhance gene-therapeutic approaches. Nevertheless, their *in vivo* feasibility has to be tested thoroughly, and their therapeutic benefit has yet to be proven. The natural or genetically modified tropism of viral vectors is another possible way to achieve a tumor-specific gene transfer. The strict dependency of retroviral vectors on cell division has been utilized to achieve a selective transfer of a suicide gene into glioblastoma cells (with few exceptions, the only dividing cell within the brain) [49, 50]. Recent experiments pointed out that AAV seems to have a natural tropism for hepatocytes, which can be utilized for liver-specific gene transfer [93].

Recombinant DNA technologies have been used to alter the natural tropism of viruses and to generate target-specific gene-transfer vehicles. Retroviruses, for example, have been modified by pseudotyping with human immunodeficiency virus envelope proteins [94, 95] or by substituting *env* domains with antibody fragments [96]. The specificity of adenoviral vectors was modified by coupling ligands for target-specific receptors to anti-fiber [97] or anti-knob [98] antibodies or including receptor-binding domains into the fiber proteins [99]. These first results from cell culture or animal models are encouraging, but their relevance for therapeutic applications awaits proof.

Other concepts rely on a target-specific gene expression instead of a specific gene delivery. The initial finding that lytic replication of the adenovirus E1b-deletion variant ONYX-015 occurs selectively in p53 deficient cells [100, 101] is the underlying concept used to destroy (by an adenoviral infection) tumors harboring an inactivated p53 gene. The clinical potential of this concept was noted along with the prospect of rapid clinical benefits [102, 103, 104]. Unfortunately, new experiments revealed that ONYX-015 replicated independently of the p53 status in various tumor cell lines and that this virus is able to replicate in and to kill primary human cells [105]. The molecular basis for the growth differences of ONYX-015 within different cell types remains to be determined, and the therapeutic value of this virus has to be evaluated with great care. A related strategy made use of the observation that reovirus infection of human cells in culture requires an activated ras pathway. In an animal model, it was shown that the injection of reovirus into the tumor could lead to tumor regression [106]. Whether this model will have any clinical impact awaits further exploration.

Attempts to control recombinant gene expression by promoters that are responsive to exogenously applied agents are underway [107, 108] and may be important contributions to more sophisticated gene-therapy approaches. Gastrointestinal malignancies often bear a high

risk of forming multiple metastasis and, therefore, escape the surgeon's knife. Several gene therapy approaches have the potential to fight malignant cells irrespective of their location in the patient's body. A successful immunotherapy would enable immune cells migrating through the body to identify and destroy malignant cells at virtually any place. Gene-transfer vectors targeted to malignant cells may be administered systemically, and metastases could be reached without precise knowledge of their number, size and location. The treatment of those gastrointestinal malignancies bearing a poor prognosis

might particularly benefit from effective and safe therapeutic gene transfer in the future.

To date, all attempts to treat cancer by gene therapy have focused on the treatment of existing neoplasms. Some researchers have now begun to discuss the possible benefits of prophylactic gene therapy to prevent malignant transformation in the specific context of high-risk tissues [109]. Using gene replacement or antisense techniques, prophylactic approaches may add functional tumor-suppressor genes or suppress the expression of activated proto-oncogenes.

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