

Molecular therapy of gastric cancer

Review Article

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Key Words: Gastric cancer, molecular therapy, carcinogenesis, gene therapy

Abbreviations: 5-fluorouracil, (5-FU); adenomatous polyposis coli, (*APC*); ATP-binding cassette, (*ABC*); biological response modifiers, (*BRMs*); Breast cancer resistance protein/mitoxantrone resistance associated transporter, (*BCRP/MXR*); Camptothecin, (*CPT*); carcinoembryonic antigen, (*CEA*); CD/5-FC, (cytosine deaminase/5-fluorocytosine); cisplatin, (*CDDP*); cytotoxic T cells, (*CTLs*); deleted in colorectal carcinoma, (*DCC*); dendritic cells, (*DCs*); epidermal growth factor receptor, (*EGFR*); Epithelial cell adhesion molecule, (*EpCAM*); etoposide phosphate, (*EP*); extracellular matrix, (*ECM*); extracellular matrix protein 1, (*ECM1*); fragile histidine triad, (*FHIT*); hepatocyte growth factor, (*HGF*); histone deacetylase, (*HDAC*); HSK-TK/GCV, (herpes simplex virus thymidine kinase/ganciclovir); human leukocyte antigen, (*HLA*); human telomerase RNA, (*hTR*); Human telomeric repeat binding factors, (*TRFs*); hypoxia-inducible factor-1, (*HIF-1*); Intercellular adhesion molecule-1, (*ICAM-1*); Intestinal alkaline phosphatase, (*IAP*); loss of heterozygosity, (*LOH*); matrix metalloproteinases, (*MMPs*); multidrug resistance, (*MDR*); multidrug resistance gene-1/P-glycoprotein, (*MDR1/PGP*); Multidrug resistance-associated protein 1, (*MRP1*); N-Methyl-N'-nitro-N-nitrosoguanidine, (*MNNG*); Nucleotide excision repair, (*NER*); peroxisome proliferator-activated receptor, (*PPAR*); phosphatase and tensin homolog, (*PTEN*); picibanil, (*OK-432*); plasminogen activation, (*PA*); polysaccharide-K, (*PSK*); proliferating cell nuclear antigen, (*PCNA*); retinoblastoma, (*Rb*); small interfering RNA, (*siRNA*); telomerase-associated protein 1, (*TEP1*); uracil phosphoribosyltransferase/5-Fluorouracil, (*UPRT/5-FU*); vascular endothelial adhesion molecule-1, (*VCAM-1*); Vascular endothelial growth factor, (*VEGF*); vincristine, (*VCR*); Zinc ribbon domain-containing 1, (*ZNRD1*)

Received: 28 November 2003; Accepted: 15 December 2003; electronically published: December 2003

Summary

Gastric cancer is the second most common cause of cancer-related death worldwide. In Western countries, most cancer patients are diagnosed at an advanced tumor stage and therefore the overall prognosis of gastric cancer is dismal. Currently the most effective treatment of gastric cancer is surgical resection of the tumor with lymphadenectomy because gastric cancer cells, in general, have low sensitivity to chemotherapy and radiotherapy. During the past decades, much has been learnt about molecular alterations in gastric cancer, which enable us to develop new molecular therapies in gastric cancer. This review will highlight the molecular changes in gastric carcinogenesis and metastasis, as well as the ongoing molecular therapy based on the understanding of the molecular mechanisms underlying gastric carcinogenesis.

I. Introduction

Although the incidence and mortality of gastric cancer has declined during the last 50 years, it continues to be the second most common cancer and the second leading cause of cancer death worldwide (Hanahan and Weinberg, 2000). In Europe the annual incidence is 12-15 per 100000, with Portugal at the top end of this range (Hohenberger and Gretschel, 2003). Currently the most effective treatment of gastric cancer is surgical resection of the tumor with lymphadenectomy because gastric cancers are largely resistant to chemotherapy and radiotherapy. However, two-thirds of the Western gastric cancer patients are diagnosed in advanced stages, when surgery can only be palliative (Nardone, 2003). Due to its limited treatment

options and poor prognosis, gastric cancer therefore remains a major clinical challenge.

Carcinogenesis and metastasis of gastric cancer is a complex multistep malignant process, which is considered to be the result of an interplay between the host genetic profile and environmental toxic agents. Several exogenous factors are suspected to contribute to gastric carcinogenesis, including diet (such as high intake of salted and nitrated food), chemicals (such as N-methyl-N'-Nitro-N-Nitrosoguanidine, Nitric oxide, Catechol and N-methyl-N'-Nitrosourea) and infectious agents (such as infection of *Helicobacter pylori* and *Epstein-Barr Virus*) (Stadtlander and Waterbor, 1999). *H. pylori* triggers and promotes gastric carcinogenesis and represents the most

important infectious risk factor. Prevention of gastric cancer seems to be feasible through the eradication of *H. pylori* and the reduction of inflammation originating from *H. pylori* infection. However, studies during the past 10 years provide evidence that multiple genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes, as well as genetic instability, telomerase activation, neoangiogenesis, abnormalities in drug metabolism and immune response of the host may play a crucial role in the pathogenesis and progression of gastric cancer (Stadtlander and Waterbor, 1999; Yasui et al, 2001; Nardone, 2003). Based on the understanding of the molecular mechanisms underlying gastric carcinogenesis and metastasis, great hopes are pinned on the development of new targeted therapy directed at tumor-specific molecular defects in gastric cancer. These molecular strategies include direct induction of tumor cell death, reversal of tumorigenesis by correcting genetic abnormalities, enhancing tumor response rate to conventional chemo- and radiotherapy, modulation of the host immune response against tumors, and protection of normal tissue from toxic effects of anti-tumor treatment by means of drug or gene therapy. In this review, we will highlight the ongoing molecular therapies based on the molecular changes in gastric carcinogenesis and metastasis.

II. Oncogene activation and targeted therapy

Oncogenes include growth factors and growth factor receptors (i.e. *c-erbB-2*, *c-met*), signal transduction proteins (i.e. *K-ras*), nuclear transcription factors (i.e. *N-myc*, *c-fos*) and cell cycle regulation proteins (i.e. cyclin D). Abnormal activation of oncogenes by chromosomal rearrangements or gene mutation can lead to neoplastic transformation. Several oncogenes were reported to be overexpressed in gastric cancer. For example, *c-met* (a protooncogene encoding the hepatocyte growth factor receptor) and *K-sam* (a fibroblast growth factor receptor) are preferentially amplified in diffuse type gastric cancer, whereas *c-erbB-2* (an epidermal growth factor receptor) is selectively overexpressed in intestinal tumours (Stadtlander and Waterbor, 1999; Nardone, 2003). Molecular therapy targeted to oncogenes and their products include blocking oncogene expression by antisense oligonucleotides or specific hammerhead ribozymes; furthermore inhibition of oncogenes may also result from monoclonal antibodies or antagonistic drugs. Antisense therapy has been shown to reduce the expression of *c-myc* (Chen et al, 2001), *K-ras* (Song et al, 2000), *EGFR* (epidermal growth factor receptor) (Hirao et al, 1999), cyclin D1 (Chen et al, 1999) and *PCNA* (proliferating cell nuclear antigen) (Sakakura et al, 1995) in gastric cancer cells and resulted in growth inhibition and apoptosis. Tumorigenicity in the nude mice injected with antisense-treated gastric cancer cells was also decreased significantly. Reversion of the malignant phenotype in gastric cancer cells SGC7901 by *c-erbB-2*-specific hammerhead ribozyme treatment has been

reported by Bi et al. (2001). Activation of peroxisome proliferator-activated receptor (PPAR) by troglitazone, a potent and selective PPAR ligand, was shown to inhibit the growth of MKN-45 cells, a human gastric cancer cell line, through the suppression of *c-met* transcription (Kitamura et al, 1999). Antibodies directed against EGFR (MoAb 528) have been reported to result in growth suppression of human gastric cancer cells overexpressing EGFR (Teramoto et al, 1996). HGF (hepatocyte growth factor) is involved in malignant behavior of cancers as a mediator of tumor-stromal interactions, facilitating tumor invasion and metastasis. Blockade of HGF using recombinant NK4, an HGF antagonist, leads to growth inhibition of the human gastric carcinoma cell line TMK1 (Hirao et al, 2002).

III. Tumor-suppressor-gene inactivation and related therapy

Tumor suppressor genes act as negative regulators in cell cycle and cell proliferation. Inactivation of tumor suppressor genes such as *p53*, *p16*, *APC* (adenomatous polyposis coli), *DCC* (deleted in colorectal carcinoma) and *Rb* (retinoblastoma) by genetic alterations including mutation, loss of heterozygosity (LOH) of chromosomes carrying tumor suppressor genes or chromosomal rearrangements is believed to play an important role in gastric carcinogenesis (Stadtlander and Waterbor, 1999; Nardone, 2003). Mutations of *p53* have been described in around 50% of advanced gastric cancers and up to 60% of intestinal-type gastric cancers have mutation or loss of heterozygosity of the *APC* gene (Nardone, 2003). *FHIT* (fragile histidine triad) is a new suppressor gene that induces apoptosis and inhibits cell proliferation (Sard et al, 1999). In our recent study, we found that absent or reduced expression of FHIT protein is associated with poorly differentiated diffuse type of gastric cancer (Rocco et al, 2003). *PTEN* (phosphatase and tensin homolog) is another candidate tumor suppressor gene and our study has demonstrated that *PTEN* expression was reduced in gastric cancer and in the gastric mucosa of gastric cancer relatives (Fei et al, 2002). Apart from genetic alterations, epigenetic alterations, which alter the heritable state of gene expression, have drawn more and more attention in recent years. Epigenetic alterations are mediated by formation of transcriptionally repressive chromatin states around gene transcription start sites caused by methylation in normally unmethylated CpG islands in gene promoter region and histone deacetylase (HDAC) activity (Cameron et al, 1999; Baylin et al, 2001). A significant proportion of tumor-related genes, including well-characterized tumor suppressor genes (*p16^{INK4a}*, *p15^{INK4b}*, *p14^{ARF}*, *p73*, *APC*, and *BRCA1*), DNA repair genes (*hMLH1*, *GSTP1*, and *MGMT*), and genes related to metastasis and invasion (*CDH1*, *TIMP3*, and *DAPK*) have been demonstrated to be silenced by aberrant promoter hypermethylation in cancer (Esteller et al, 2001, 2002).

Previous studies have demonstrated that adenovirus-mediated transfer of the wild-type *p53* gene results in growth inhibition of gastric carcinoma cells both in vitro and in vivo through the apoptosis pathway (Ohashi et al,

1999; Tatebe et al, 1999). Two very recently published studies also used this strategy to deliver the wild-type *FHIT* gene in the treatment of forestomach tumors in mice (Ishii et al, 2003) and *p16^{INK4A}* gene in the treatment of gastric cancer cells (Jeong et al, 2003); both studies obtained promising results. The other potential molecular therapy is the so-called “transcriptional therapy”, using DNA demethylating drugs (i.e. 5'-azadeoxycytidine, Procainamide) and histone hyperacetylating drugs (i.e. 4-Phenylbutyrate, Trichostatin A) to reactivate silenced tumor suppressor genes, which are considered very promising in the treatment of various cancers, including gastric cancer (Jung, 2001; Chiurazzi and Neri, 2003).

IV. Apoptosis targeted therapy

Apoptosis plays a fundamental role in a multicellular organism. In contrast to necrosis, it ensures a rapid and complete removal of cells that are no longer required or dangerous for the organism (Raff, 1998). From a biological point of view, the chronic imbalance between cell proliferation and apoptosis is an early step of gastric carcinogenesis, as in other tumors (Hanahan and Weinberg, 2000). Induction of apoptosis and cell cycle arrest is one of the main antineoplastic mechanisms. As we described previously, oncogene blockage or tumor suppressor gene reactivation therapy usually lead to the growth inhibition of gastric carcinoma through apoptosis related pathways. Moreover, various types of anti-neoplastic agents also achieve therapeutic effects by apoptosis induction. Camptothecin (CPT), a inhibitor of topoisomerase I, is effective in the treatment of certain solid tumors; treatment with CPT effectively inhibits the growth of the human gastric cancer SIIA in nude mice; the mechanism involved is considered to be induction of apoptosis mediated by up-regulation of *p53*, *p21Waf1/Cip1*, and *p27Kip1* and the down-regulation of *Bcl-2* and *Bcl-XL* (Litvak et al, 1999). Induction of apoptosis by oral anti-neoplastic agents, such as tegafur and uracil (UFT, a combined preparation of 1 mol tegafur and 4 mol uracil) was also observed in human gastrointestinal tumor xenografts generated in nude mice (Oki et al, 1998). One recent study showed that SC-236, a *COX-2*-specific inhibitor, had anti-proliferative effects in gastric cancer cells by inducing apoptosis through a protein kinase C- dependent pathway (Jiang et al, 2002). Muller et al (1998) demonstrated that multiple anticancer drugs, including cisplatin, mitomycin, methotrexate, mitoxantrone, doxorubicin, and bleomycin could induce p53-dependent apoptosis mediated by the CD95 (APO-1/Fas) receptor/ligand system in different cancer cell lines. *E2F-1* is a transcription factor that regulates cell cycle progression into S-phase. Deregulation of *E2F-1* activity has been associated with cellular commitment to apoptosis. Adenovirus-mediated *E2F-1* gene transfer together with treatment using cyclin-dependent kinase inhibitors resulted in an enhanced apoptotic response in human gastric carcinoma cells (Atienza et al, 2000). Other apoptosis-related genes have also been described in targeted gastric cancer therapy. SC-1, an apoptosis cell-

surface receptor, is associated with diffuse type gastric tumors (Vollmers et al, 1997); administration of a monoclonal antibody against SC-1 induces apoptosis and inhibits proliferation of gastric cancer cells (Vollmers et al, 1998). Caspase-8 and Caspase-3 are members of the cysteine protease family that modulate apoptosis induced by a variety of cell death signals. Transfection of caspase-8 and caspase-3 could augment apoptosis and inhibit peritoneal dissemination of human gastric carcinoma cells (Nishimura et al, 2001; Fu et al, 2003).

V. Anti-metastasis therapy

Advanced gastric cancer is often accompanied by metastasis to the lymph nodes, liver, peritoneum or other organs, resulting in a high mortality rate. With regard to cancer invasion and metastasis, molecular alterations in cell-cell or cell-matrix interactions and angiogenesis are considered to be very important.

Several extracellular proteolytic systems are involved in the formation of metastasis by extracellular matrix degradation and the most thoroughly investigated systems are the plasminogen activation (PA) system and the matrix metalloproteinases (MMPs) (Almholt and Johnsen, 2003). MMPs are zinc-dependent proteases, which are active at physiological pH and are either located at the cell membrane (MT-MMP) or are secreted. The MMP-family constitutes over 21 proteases that are capable of selectively digesting a wide spectrum of both extracellular matrix (ECM) and nonmatrix proteins. MMPs play a critical role in tumor growth, angiogenesis, and metastatic processes (Li and Anderson, 2003). In gastric cancer, up-regulation of MMP-1, MMP-2, MMP-7 and MMP-9 have been reported to be associated with peritoneal dissemination and lymph node metastasis (Murray et al, 1998; Yonemura et al, 2000; Monig et al, 2001). Matsuoka et al. (2000, 2001), have reported the inhibition of invasion and lymph node metastasis of gastrointestinal cancer cells by R-94138, a MMP inhibitor specific to MMP-2 and MMP-9, meanwhile, the inhibition of peritoneal dissemination in human gastric cancers by MMP-7-specific antisense oligonucleotide has been reported by Yonemura et al. (2001).

Adhesion molecules participate in multiple steps in cancer development including dissociation and release of gastric cancer cells from their primary cancer nests, lodging of malignant cells between endothelial cells and subsequent adhesion to the extracellular matrix of distant host tissues leading to the manifestation of metastatic nodules. Several adhesion molecules were found to be up or down regulated in gastric cancer and provide new targets for molecular therapy. E-cadherin, - and - catenin form the cadherin-catenin complex and are critical for establishing intercellular adhesion. Reduction or loss of E-cadherin expression has been described in gastric cancer, and germline mutations of the E-cadherin gene have been detected in 50-70% of diffuse-type gastric cancers and are responsible for a small subset of familial gastric cancers (Caldas et al, 1999). We and others have reported the frequent down-regulation of - and -catenin in primary and metastatic gastric

cancer (Yu et al, 2000; Ebert et al, 2003). E-cadherin gene mutations typically affect the extracellular portion of the homophilic receptor and cancer-specific monoclonal antibodies against the E-cadherin mutational hot spot region are now available. After linking to toxins, drugs or radiolabelling, the E-cadherin mutation-specific antibodies could serve as very specific agents to treat gastric cancer (Becker and Höfler, 2001). Intercellular adhesion molecule-1 (*ICAM-1*) is another down-regulated adhesion molecule, which contributes to lymph node metastasis of gastric cancer. Sunami et al. (2000) reported that *ICAM-1* gene transfection inhibited lymph node metastasis of human gastric cancer cells both in vitro and in vivo; moreover, the adhesion and cytotoxic effect of peripheral blood mononuclear cells were significantly increased against cancer cells with high *ICAM-1* expression (Tanaka et al, 2002). Other adhesion molecules, including members of the integrin receptor family, the laminin binding protein, E-selectin, vascular endothelial adhesion molecule-1 (*VCAM-1*) and CD44 receptor have been found to be up-regulated during the development of gastric cancer metastasis (Streit et al, 1996; Ura et al, 1998; Gulubova, 2000). Neutralizing antibodies directed against integrin 2 or 1 reduced gastric cancer peritoneal dissemination in nude mice (Kawamura et al, 2001), while adhesion polypeptides, which block the binding of integrins to the ECM also resulted in inhibition of peritoneal implantation of gastric cancer cells (Matsuoka et al, 1998). Epithelial cell adhesion molecule (*EpCAM*) is expressed in gastric cancer but not in normal gastric epithelium. Selective gene delivery toward gastric adenocarcinoma cells via *EpCAM*-targeted adenoviral vectors has resulted in a favourable tumor-over-normal tissue transduction ratio thereby increasing specificity of gastric cancer gene therapy (Heideman et al, 2002).

The growth of solid tumors and the formation of metastases largely depend on angiogenesis. Both tumor cells and host cells secrete a variety of factors to stimulate angiogenesis. Thus, angiogenesis is another potential target for molecular therapy of gastric cancer. Vascular endothelial growth factor (*VEGF*), hypoxia-inducible factor-1 (*HIF-1*) and extracellular matrix protein 1 (*ECM1*) are angiogenic factors implicated in hematogenous invasion or metastasis in gastric cancers (Kakeji et al, 2002; Wang et al, 2003). Repeated intraperitoneal transduction of a soluble *flt-1* gene, one of the *VEGF* receptors, using HVJ-cationic liposomes suppressed peritoneal metastases of gastric cancer cells (Mori et al, 2000). *YC-1*, 3-(5'-hydroxymethyl-2'-furyl)-1-benzylindazole, a *HIF-1* inhibitor, could block angiogenesis and inhibit stomach tumor growth in mice (Yeo et al, 2003). Angiostatin is a circulating inhibitor of angiogenesis generated by proteolytic cleavage of plasminogen (Lucas et al, 1998). Angiostatin is also an inhibitor of tumor angiogenesis which targets the proliferating tumor vasculature and induces regression of experimental tumors and enhances the antitumor effects of radiation therapy (Hari et al, 2000). Wu et al. (2003) reported that angiostatin gene transfection inhibited angiogenesis and tumorigenesis of human gastric cancer in

nude mice. Marimastat, a broad-spectrum MMP-inhibitor inhibits peritoneal dissemination of human gastric cancer cells through inhibition of tumor angiogenesis by down-regulation of gelatinases in SCID mice (Wada et al, 2003).

VI. Telomerase inhibition therapy

Telomeres are DNA-protein structures that cap linear chromosomes and are essential for maintaining genomic stability and cell phenotype. The stabilization of telomeres is required for continuous cell proliferation as well as for the attainment of immortality in tumor cells. Telomere length is regulated by the telomerase and other related proteins. Telomerase is an RNA-dependent DNA polymerase that synthesizes TTAGGG telomeric DNA onto chromosome ends to compensate for sequence loss during DNA replication. In adults, telomerase is down-regulated in most somatic tissues while it remains active in germs cells. Activity of telomerase has been found in almost all human tumors and its activation seems to be a mandatory step in carcinogenesis (Krupp et al, 2000). Three components of the human telomerase complex, including human telomerase RNA component (*hTR*), human telomerase reverse transcriptase (*hTERT*), and telomerase-associated protein 1 (*TEP1*) have been cloned; meanwhile, other factors, which regulate telomere length, have also been identified more recently. Human telomeric repeat binding factors (*TRFs*) *TRF1* and *TRF2*, and human telomere-associated protein *TIN2* are negative regulators of telomere length, while tankyrase and *Rap1* act as positive regulators (Kim et al, 1999; Li et al, 2000; Cook et al, 2002). Reactivation of telomerase (Hiyama et al, 1995; Kakeji et al, 2001; Nowak et al, 2003; Yoo et al, 2003), down-regulation of *TRF1*, *TRF2* and *TIN2* (Miyachi et al, 2002; Yamada et al, 2002), and frequent loss of heterozygosity on chromosome 10p15, a putative telomerase repressor/senescence gene locus (Hiyama et al, 2003) have been reported recently in gastric cancer. This indicates that the maintenance of telomere length may play a significant role in the tumorigenesis of gastric cancer and may reflect the malignant potential of the tumor. Therefore, telomerase inhibitors are attractive tools for gastric tumor therapy. Recently, several groups were able to induce cell cycle arrest and to inhibit cell growth in gastric cancer cells by antisense telomerase RNA (*anti-hTR*) treatment. This treatment targets rather specifically and selectively cancer tissue but not normal tissue making it highly attractive for the treatment of gastric cancer (Naka et al, 1999; Yang et al, 2002; Wong et al, 2003).

VII. Gene directed chemotherapy

Although gastric cancers are largely resistant to chemotherapy, based on the knowledge of the molecular mechanisms underlying gastric carcinogenesis and anticancer drug metabolism, a new strategy termed "gene-chemotherapy" has been introduced more recently in gastric cancer therapy. One type of gene-chemotherapy is aimed at reversal of the chemoresistance of cancer cells in chemotherapy, and the other type was previously called "cytotoxic gene therapy" or "suicide gene therapy".

The phenomenon of drug resistance frequently

occurs in gastric cancer chemotherapy and results in the failure of treatment. Chemoresistance of cancer cells is due to abnormal alterations of oncogenes, tumor suppressor genes, apoptosis-related genes and specific or multidrug resistance (MDR) genes. Gene therapy targeted at these chemoresistance-related genes can reverse tumors with drug-resistance phenotype to drug-sensitive and thereby enhance the effect of chemotherapy. *c-erbB-2* expression in gastric cancer is one of the factors related to cisplatin sensitivity and anti-*c-erbB-2* antisense oligonucleotides induce increased sensitivity to cisplatin (Funato et al, 2001). Transfer of *Bax*, an important proapoptotic gene, could reduce growth rate and increase sensitivity to 5-fluorouracil (5-FU) and cisplatin (CDDP) in human gastric cancer cells both in vitro and in vivo (Komatsu et al, 2000; Kim et al, 2001). However, *Bcl-2* gene, a homologue of *Bax*, counteracts the apoptosis induction activity of *Bax*. *Bcl-2* treatment with antisense oligonucleotides (G3139) could therefore chemosensitize human gastric cancer cells to cisplatin, as demonstrated in a SCID mouse xenotransplantation model with downregulation of *Bcl-2* expression and increased apoptosis (Wacheck et al, 2001). DNA repair is another important modulator of resistance to platinum-based anticancer chemotherapy. Nucleotide excision repair (NER) is the DNA repair pathway responsible for the repair of cisplatin-DNA damage. *ERCC1* is one critical gene within NER and in human gastric cancer *ERCC1* is a useful marker for clinical drug resistance when platinum-based systemic chemotherapy is utilized (Reed, 1998). *ZNRD1* (Zinc ribbon domain-containing 1) is a gene associated with vincristine (VCR) resistance in gastric cancer cells; *ZNRD1* antisense treatment sensitizes drug resistant gastric cancer cells to VCR treatment as demonstrated in a recent study (Zhang et al, 2003). Multidrug resistance-associated protein 1 (MRP1) and multidrug resistance gene-1/P-glycoprotein (MDR1/PGP) are cell membrane drug efflux pumps related to the classical MDR phenotype of gastric cancer (Stein et al, 2002). For reversal of MDR1 gene-dependent multidrug resistance, Nieth et al (2003) used two small interfering RNA (siRNA) constructs to inhibit MDR1 expression by RNA interference in human gastric cancer cells, which resulted in a significant reduction of resistance against daunorubicin. Another interesting strategy is transduction of the *MDR-1* gene into haematopoietic stem cells with the aim of both reducing bone marrow toxicity from chemotherapeutic agents, and facilitating the use of more intensive and high-dose treatment protocols (Szlosarek and Dalglish, 2000), although it has not been explored in gastric cancer chemotherapy. Breast cancer resistance protein/mitoxantrone resistance associated transporter (BCRP/MXR) is a new member of the superfamily of ATP-binding cassette (ABC) transporters associated with resistance to mitoxantrone and anthracyclines in a multidrug resistant phenotype of gastric cancer (Ross et al, 1999; Stein et al, 2002). Modulation of the atypical multidrug-resistant phenotype of gastric cancer cells by a hammerhead ribozyme directed against the ABC transporter BCRP/MXR/ABCG2 was reported by Kowalski et al. (2002).

The strategy of cytotoxic gene therapy or suicide gene therapy involves the transduction of tumor cells with a foreign enzyme, following administration of a prodrug. The transduced enzyme catalyses the formation of toxic molecules, which induces tumor cell death. By using tissue-specific promoter, the enzyme transduction can be targeted at special tumor tissues. The most frequently investigated enzyme/prodrug systems in cytotoxic gene therapy of gastric cancer are the HSK-TK/GCV (herpes simplex virus thymidine kinase/ganciclovir) system, the CD/5-FC (cytosine deaminase/5-fluorocytosine) system, the UPRT/5-FU (uracil phosphoribosyltransferase/5-Fluorouracil) system and very recently the IAP/EP (intestinal alkaline phosphatase/etoposide phosphate) system. Ganciclovir is a widely used non-cytotoxic antiviral drug; after phosphorylation by HSK-TK, it is converted to a cytotoxic drug; transfection of HSK-TK followed by ganciclovir treatment has been shown to be effective in gastric cell lines both in vitro and in vivo (Tanaka et al, 1996; Terazaki et al, 2003). Moreover, in situ gene transfer of HSK-TK followed by ganciclovir treatment has resulted in degeneration of cancer tissue and fibrosis after necrosis and apoptosis in both primary tumors and lymph node metastases in N-ethyl-N'-nitro-N-nitrosoguanidine (ENNG) induced rat and dog gastric cancer models (Matsukura et al, 1999; Okino et al, 2001). These promising results may be useful for the application of suicide gene therapy in humans. Similar to ganciclovir, the non-cytotoxic fluorocytosine can also be converted to cytotoxic fluorouracil under the activation by the cytosine deaminase. Transduction of gastric cancer cells with the *Escherichia coli* cytosine deaminase followed by 5-fluorocytosine prodrug therapy has been explored both in vitro and in vivo with promising results (Lan et al, 1996, 1997; Ueda et al, 2001). 5-FU, a widely used chemotherapeutic agent, has a limited effect in the treatment of human solid tumors. Transduction of the *E. coli* UPRT gene results in marked sensitization of gastric cancer cell lines to low concentration of 5-FU both in vitro and in vivo (Kanai et al, 1998; Inaba et al, 1999). Intestinal alkaline phosphatase (IAP) is capable of converting a relatively non-cytotoxic prodrug, etoposide phosphate (EP), into etoposide with a significant antitumor activity. A very recent study demonstrated that the prodrug-converting system by membrane-bound IAP gene and the EP prodrug is useful in gastric cancer treatment (Kim et al, 2003). Since about 40% of gastric cancers express CEA (carcinoembryonic antigen), the CEA promoter is frequently used in different cytotoxic gene therapy systems of gastric cancer for selective gene targeting (Lan et al, 1996, 1997; Tanaka et al, 1996; Ueda et al, 2001).

VIII. Immunotherapy

Gastric cancer cells, in general, have a low sensitivity to chemotherapy and a low immunogenicity related to stimulation of immune competent cells. However, a new method including biochemical modulation and nonspecific immunopotentialization with biological response modifiers (BRMs) has permitted to

augment the clinical efficacy of immunochemotherapy in gastric cancer (Toge, 1999). Adjuvant immunochemotherapy of gastric cancer has been conducted in patients with malignant effusion using BRMs, including polysaccharide-K (PSK) and picibanil (OK-432). OK-432 is a lyophilized, heat-inactivated, penicillin treated powder of a low virulence strain of *Streptococcus pyogenes* A3. OK-432 strongly stimulates the cellular immune-response, especially of natural killer cells and macrophages, and induces the production of interleukins, interferons and tumor necrosis factor; PSK is a protein-bound polysaccharide extracted from the mycelia of *Coriolus versicolor* (strain CM-101) of *Basidiomycetes*. PSK could restore cancer-related immunosuppression by competing with soluble immunosuppressive factors as well as stimulate the cellular immune-response (Toge, 1999). Combining PSK or OK-432 with chemotherapy reagents in the treatment of gastric cancer has demonstrated to be effective in several clinical trails (Toge, 1999).

Genetic immunotherapy is another area of active research. Administration of an adenovirus vector expressing IL-6 induced CD⁸⁺ cytotoxic T-lymphocytes specific for gastric cancer cells from the precursor human T-lymphocytes *in vivo*, and thereby inhibited growth and metastasis of autologous human tumors (Tanaka et al, 1997). In another study, tumorigenicity of IL-2 producing gastric cancer cells was significantly reduced in the CD³⁴⁺ reconstituted but not in the non-reconstituted mice, whereas transduction of IL-6 did not affect tumorigenicity, irrespective of the reconstitution status of the mice (Tagawa et al, 1998).

Apart from the nonspecific immunotherapy, novel cancer vaccines have been designed recently and provide specific immune treatment for gastric cancer. Gastrin is an important hormone associated with gastric acid production and growth of gastrointestinal mucosa. The hormone is processed in several steps and the 17-aminoacid product, G17, appears to be important in cancer growth. G17DT is an immunogen created by attaching G17 to the highly immunogenic diphtheria toxin. A phase II trial of the

antigastrin agent G17DT has shown promising results in the treatment of stomach cancer (Watson and Gilliam, 2001; Kerr, 2002). In a pilot clinical trial gastric cancer patients immunized with a cancer vaccine composed of EGF linked to a carrier protein developed antibodies against EGF (Gonzalez et al, 1998). MG7 is a gastric cancer specific tumor associated antigen. The oral DNA vaccine against the MG7-Ag epitope of gastric cancer can induce significant humoral immunity and partially protect against tumor development in mice (Guo et al, 2003). HER-2/neu (*c-erbB-2*)-derived peptides are naturally processed as tumor-associated antigens and are recognized by tumor-specific, human leukocyte antigen (HLA)-A2-restricted cytotoxic T cells (CTLs) in gastric cancer. A phase-1 vaccination trial in gastric cancer patients using dendritic cells (DCs) pulsed with the immunodominant HER-2/neu (p369) peptides has been reported with promising results (Kono et al, 2002).

IX. Conclusions

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 (Table 1). Using new technologies including cDNA microarray and proteomics, a full understanding of the molecular processes underlying gastric carcinogenesis may lead to further development and design of new molecular therapies "tailored" to a single subject with gastric cancer.

Acknowledgements

M. Ebert is supported by a grant from the Deutsche Forschungsgemeinschaft (Eb 187/4-1) and a Heisenberg-Stipend (Eb 187/5-1).

Table 1. Summary of targets and strategies for molecular therapy in gastric cancer

Target molecules	Strategies	Effects
Oncogenes		
<i>c-myc</i>	Antisense oligonucleotides	Growth inhibition and apoptosis
<i>K-ras</i>	Antisense oligonucleotides	Growth inhibition and apoptosis
<i>PCNA</i>	Antisense oligonucleotides	Growth inhibition and apoptosis
cyclin D1	Antisense oligonucleotides	Growth inhibition
<i>EGFR</i>	Antisense oligonucleotides/monoclonal antibodies	Growth inhibition
<i>c-erbB-2</i>	Hammerhead ribozyme treatment	Malignant phenotype reversion
<i>c-met</i>	Troglitazone, a PPAR ligand	Growth inhibition
HGF	NK4, an HGF antagonist	Growth inhibition
Tumor-suppressor-genes		
<i>p53</i>	Gene transduction	Growth inhibition and apoptosis

<i>FHIT</i>	Gene transduction	Growth inhibition and apoptosis
<i>p16^{INK4A}</i>	Gene transduction	Growth inhibition and apoptosis
Group of genes silenced by aberrant DNA methylation	DNA demethylating drugs	Reactivation of tumor suppressor genes
Group of genes silenced by lack of histone acetylation	Histone hyperacetylating drugs	Reactivation of tumor suppressor genes
Apoptosis-related molecules		
<i>E2F -1</i>	Gene transduction	Growth inhibition and apoptosis
SC-1	Monoclonal antibody	Proliferation inhibition and apoptosis
Caspase-8	Gene transduction	Metastasis inhibition and apoptosis
Caspase-3	Gene transduction	Metastasis inhibition and apoptosis
Metastasis-related molecules		
MMP-2	R-94138, a MMP inhibitor	Metastasis inhibition
MMP-9	R-94138, a MMP inhibitor	Metastasis inhibition
MMP-7	Antisense oligonucleotides	Metastasis inhibition
E-cadherin	Monoclonal antibody	Adhesion-molecule dependent targeting
<i>ICAM-1</i>	Gene transduction	Metastasis inhibition
Integrin	Neutralizing antibody/blocking peptides	Metastasis inhibition
EpCAM	Targeted gene transduction	Targeted gastric cancer gene therapy
VEGF	flt-1 gene transduction	Angiogenesis inhibition
HIF-1	YC-1, a HIF-1 inhibitor	Angiogenesis inhibition
Angiostatin	Gene transduction	Angiogenesis inhibition
Gelatinases	Marimastat, a MMP inhibitor	Angiogenesis inhibition
Telomerase		
hTR	Antisense oligonucleotides	Growth inhibition and cell cycle arrest
Chemoresistance related genes		
<i>c-erbB-2</i>	Antisense oligonucleotides	Sensitization to cisplatin
Bax	Gene transduction	Apoptosis, sensitization to 5-fluorouracil and cisplatin
<i>Bcl-2</i>	Antisense oligonucleotides	Apoptosis, sensitization to cisplatin
<i>ZNRD1</i>	Antisense oligonucleotides	Sensitization to vincristine
MDR1	Small interfering RNA	Sensitization to daunorubicin
BCRP	Hammerhead ribozyme treatment	Sensitization to mitoxantrone and anthracyclines
Suicide genes		
<i>HSK-TK</i>	Gene transduction	Ganciclovir phosphorylation
<i>CD</i>	Gene transduction	Fluorocytosine conversion
<i>UPRT</i>	Gene transduction	Sensitization to 5-fluorouracil
<i>IAP</i>	Gene transduction	Etoposide phosphate conversion
Immunogenetic molecules		
<i>IL-6</i>	Gene transduction	Non-specific immunity
<i>IL-2</i>	Gene transduction	Non-specific immunity
<i>HER-2/neu</i>	Cancer vaccine	Specific immunogenicity
EGF	Cancer vaccine	Specific immunogenicity
Gastrin 17	Cancer vaccine	Specific immunogenicity
MG7	Cancer vaccine	Specific immunogenicity

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