

## Review

# Recent advances in the treatment of colon cancer

R. Xu<sup>1,2</sup>, B. Zhou<sup>2</sup>, P.C.W. Fung<sup>3</sup> and X. Li<sup>1,2</sup>

<sup>1</sup>Institute of Molecular Medicine, Huaqiao University, Fujian, China,

<sup>2</sup>Gene Therapy Laboratory, GRC and <sup>3</sup>Department of Medicine, University of Hong Kong, Hong Kong

**Summary** Colorectal cancer is one of the leading causes of cancer-related deaths worldwide. Although surgical resection is still the only treatment capable of curing colon cancer, adjuvant therapy continues to play an important role in preventing recurrence and metastasis. In recent years remarkable progress has been made in the treatment of colon cancer. This review discusses recent advances in adjuvant therapy for colon cancer, including chemotherapy, immunotherapy, antiangiogenic therapy and apoptosis induction. In the meantime, molecular therapy is also elucidated in the above methods. All these new advances will provide new promises for patients of colon cancer.

**Key words:** Colon cancer, Chemotherapy, Immunotherapy, Apoptosis, Angiogenesis

## Introduction

Colorectal cancer is the second-leading cause of cancer-related deaths in Europe and the USA. It ranks second in incidence to lung cancer in men and breast cancer in women. More than 900,000 new cases of colorectal cancer are diagnosed each year and colorectal cancer accounts for nearly 500,000 cancer deaths worldwide annually (Parkin et al., 2001). There is no difference in incidence between men and women, and this kind of cancer is prevalent in individuals aged over fifty.

Colon cancer often results from a combination of genetic predisposition and environmental factors. Hereditary risk contributes to approximately 20% of cases (Narayan and Roy, 2003). The main inherited predisposition syndromes are familial adenomatous polyposis (FAP), hereditary nonpolyposis colorectal cancers (HNPCC), and other types of tumor with a familial history (Guanti and Bukvic, 2000). Environmental factors account for 80% of cases. Such

factors include a diet low in fibre, vegetables, and folate and high in fat, red meat; heavy alcohol consumption; a sedentary occupation; and cigarette smoking (Campos et al., 2005). Although colorectal cancers may appear at different times and for different reasons, they share a common random pathway from normal epithelium through polyp to carcinoma (Fearon and Vogelstein, 1990). In this process a number of genetic changes are observed, including the inactivation of the tumor suppressor genes and the activation of specific oncogenes. Molecular biological studies have shown mutations of p53, Apc, k-ras and/or changes in proteins such as APC and DNA microsatellite instability or loss of heterozygosity (Gonciarz et al., 2004). Mutations are present as inherited germline defects or arise in somatic cells secondary to environmental insult, which continues to contribute to the development of colon tumor.

Surgical resection remains the only curative treatment for colon cancer yet developed, but this treatment is often unsatisfactory, if the disease presents at an advanced stage or metastases develop after attempted curative resections. The most important pathological prognostic factor after surgery is the disease stage. Patients with stage II disease, where tumours have invaded through the muscularis propria or serosa, have a 5-year survival rate of approximately 75%. By contrast, the survival rate of stage III patients with lymph node metastases varies between 25% and 60%, depending on the number of positive nodes (DeVita et al., 2001). Hence, different types of adjuvant treatments should be developed as auxiliary weapons for surgery in order to eradicate potential cancer cells before they become visible and begin to harm the patient.

## Chemotherapy

The current standard protocol for colon cancer, which is known to significantly reduce relapse rates and risks of dying from resected colon cancer, involves 6 months of adjuvant fluorouracil (5-FU) combined with leucovorin. This protocol significantly improves the 3-year disease-free survival rate. However, only 26% of patients respond (Wils et al., 2001). Drug resistance is a

major problem that limits the effectiveness of chemotherapy, and its systemic toxic effects make it difficult for some patients to tolerate.

Multidrug resistance (MDR) is a phenomenon by which cancer cells evade the cytotoxic effects of chemotherapeutic agents. It may occur through different mechanisms, but it often correlates with the overexpression of membrane or intracellular molecular protein, resulting in increased drug efflux, decreased drug influx, drug inactivation, alterations in drug targeting, processing of drug-induced damage, or evasion of apoptosis (Longley and Johnston, 2005). Overexpression of glucosylceramide synthase and P-glycoprotein in cancer cells were connected with resistance to natural product chemotherapy. The overexpression of P-glycoprotein causes drug efflux, and reduces the efficiency of chemotherapy (Gouaze et al., 2005). Several attempts have been made to reduce multidrug resistance in cancer cells. Reduction of the doxorubicin efflux rate and increase in the drug accumulation in HT29 and HT29-dx cells by induction of NO synthesis reversed doxorubicin resistance in human cancer (Riganti et al., 2005). Other investigators have attempted to elucidate the mechanism of multidrug resistance. Penuelas et al. (2005) screened the gene expression profile in HT29 human colon cancer cell resistance to methotrexate (MTX) and found that IMPDH2, IMPCH and survivin were up-regulated, while topoisomerase I and vimentin were down-regulated. Functional analyses revealed that inhibition of IMPDH or TOP1 activity, antisense treatment against survivin, or overexpression of vimentin, sensitized resistant HT29 cells to MTX. A recent study has reported that 5-fluorouracil-resistant human colon cancer cells overexpressed the proteins Bcl-XL, Bcl-Xs, and Bik, knockdown of Bcl-XL protein expression by Bcl-XL-specific small interfering RNA could inhibit proliferation more effectively in 5-FU-resistant cells. Transfection of human metastatic colon cancer cells with kinase-defective mutants of c-Src led to a decrease in Bcl-XL and an increase in oxaliplatin- and Fas-induced apoptosis (Zhu et al., 2005).

One of the major goals for chemotherapy is to target tumor cells with toxic agents in a selective and specific manner, avoiding damage to normal tissues. Enzyme-prodrug systems are used to localize the toxic drug effects to tumor cells, and destroy tumors and metastasis but not normal tissues. Gene therapy offers the possibility of this targeted treatment, which reduces the systemic effects of chemotherapy. GDEPT (gene-directed enzyme prodrug therapy) involves gene transfer of an enzyme into tumor cells, which converts an inactive prodrug into a toxic metabolite, leading to cell death. An important feature of enzyme-prodrug systems is their 'bystander effect', whereby surrounding cells (not expressing the enzyme) are also killed by active metabolites. The enzyme expression can be genetically controlled or its delivery targeted to ensure tumor selectivity (Dachs et al., 2005). Oosterhoff et al. (2005)

constructed a replication-deficient adenoviral vector Ad.C28-sCE2 containing a fusion gene encoding a secreted form of human liver CE2 targeted to the surface antigen epithelial cell adhesion molecule (EpCAM), which is highly expressed in most colon carcinoma cells. By targeting CE2 to EpCAM, Ad.C28-sCE2-transduced colon carcinoma cells expressed and secreted active CE (the enzyme carboxylesterase) locally in the tumor, and CPT-11 was converted into SN-38, which inhibited the growth of colon cancer without toxicity (Oosterhoff et al., 2005). Intratumoral injection of an adenovirus vector encoding *Escherichia coli* nitroreductase driven by CTP1 efficiently sensitized SW480 xenografts to the prodrug CB1954, whereas systemic vector and prodrug administration produced no apparent signs of toxicity (Lipinski et al., 2001).

### Immunotherapy

Immunotherapy is the third method for colon cancer treatment. A number of approaches using antibodies and vaccines as adjuvant therapy are being studied.

Colon tumor cells can express TAA, which is able to activate the immune system. mAbs directed against tumor antigens can opsonize tumor cells and promote their elimination either by activation of cellular immune effectors like NK cells (ADCC) or by activation of the complement cascade (CDC). Edrecolomab (monoclonal antibody 17-1A), a murine monoclonal antibody that recognizes the epithelial cell-adhesion molecule known as Ep-CAM, appears to be highly effective in treating micro-metastatic disease (Schwartzberg, 2001). In a study of 189 patients with resected stage III colorectal cancer, treatment with edrecolomab resulted in a 32% increase in the overall survival rate, and a decrease in the tumor recurrence rate of 23% (Riethmuller et al., 1998). But mAb may also clear circulating tumor cells that have greater access to immune effector cells, and is ineffective against cancer cells already residing within solid tissues. To increase the anti-tumor efficacy of mAb, Bi-specific antibodies have been engineered. Anti-CD3/CEA or anti-CD3/Ep-CAM bi-specific antibodies can cross-link T cells to colon cancer cells. Furthermore, anti-CEA /B7 can convert B7-negative tumors into B7-positive tumors, provide both functional and tumor-specific signals 1 and 2 for the activation of primary human T cells, and trigger their cytotoxic activity in a tumor-specific way (Mack et al., 1995; Holliger et al., 1999).

Tumor cells can serve as vaccines to augment anti-tumor immunity after irradiation with x-rays to prevent their outgrowth *in vivo*. The effect is limited, however, though it can be improved by increasing immunogenicity of whole-cell vaccines based on the use of nonspecific immunostimulants such as bacillus Calmette-Guerin (BCG) and corynebacterium parvum, or on simultaneous gene-transduction of co-stimulatory molecules (B7, CD40L) and multiple cytokines (IL-2, IL-12, GM-CSF) (Parmiani et al., 2000). A recent phase

III clinical trial in colon cancer with autologous tumor cell-BCG vaccine demonstrated that OncoVAX® in an adjuvant setting significantly prolongs recurrence-free interval (57.1% relative risk reduction) and significantly improves 5-year overall survival and recurrence-free survival in Stage II colon cancer patients. No statistically significant prognostic benefits were achieved in Stage III patients (Uyl-de Groot et al., 2005). One of the mechanisms by which tumor cells evade the immune system is the lack of proper antigen-presenting cells. Improvement in host immunity against tumor cells can be achieved by promoting the differentiation of dendritic cells (DCs) from immature myeloid cells that accumulate in the bone marrow and lymphoid organs. Different approaches are now being explored. Some researchers are trying to target DC in vitro with tumor-derived RNA, while others are trying to load other recombinant TAA (Saha et al., 2004; Chu et al., 2005). Vaccination of antigen presenting cells can present endogenous tumor antigens, activate CTL, and effectively induce specific anti-tumor immunity. Endothelium also can serve as a vaccine, and induce an autoimmune response targeting tumor angiogenesis. Okaji et al. immunized BALB/c mice with a vaccine of glutaraldehyde-fixed murine hepatic sinusoidal endothelial cells (HSEs) in a lung metastasis model of Colon-26 cancer. Vaccination with autologous HSEs induced both preventive and therapeutic anti-tumor immunity, which significantly inhibited the development of metastases (Okaji et al., 2004).

The T cell is the effector cell in anti-tumor immune response. T cells require 2 distinct signals for optimal activation, and the genetic modification of T cells ex vivo and their re-infusion into cancer patients has recently attracted considerable attention. Recent studies revealed that gene-engineered T cells expressing chimeric single-chain (scFv) receptors were capable of co-delivering CD28 costimulation and T cell receptor zeta chain (TCR-zeta) activation signals, which after reacting with the ErbB2 tumor-associated antigen of colon cancer produced high levels of cytokines, proliferated vigorously, and mediated lysis of ErbB2(+) tumors in an antigen-specific manner. Most importantly, dual specific T cell delayed the growth of subcutaneous ErbB2(+) human tumors after systemic administration (Teng et al., 2004).

### Anti-angiogenesis

Anti-angiogenic therapy is a promising approach for colon cancer therapy, as it is less toxic than conventional chemotherapy and has a lower risk of drug resistance. Furthermore, anti-angiogenic agents can also transiently 'normalize' the abnormal structure and function of tumor vasculature to make it more efficient for drug delivery and increase the efficacy of conventional therapies (Jain, 2005). In February 2004, the FDA approved Avastin for the treatment of patients with colon cancer. Inhibition of angiogenesis has now become the

fourth modality of anti-cancer therapy.

Cancer cells depend on angiogenesis to obtain nutrients and oxygen for their outgrowth and metastasis. Tumor-induced angiogenesis involves neo-vascularization that is regulated by angiogenic and anti-angiogenic factors released from tumor cells and surrounding stromal cells. This process includes the vascular endothelial cell's migration, adhesion and invasion to extracellular matrix (ECM) and its proliferation. Adhesion molecules on the endothelial cell surface play crucial roles in the regulation of each process, and the integrins  $\alpha v \beta 3$  and  $\alpha v \beta 5$  appear to be most closely associated with tumor angiogenesis (Brooks et al., 1994; Friedlander et al., 1996). To block the function of integrin, monoclonal antibodies to integrin  $\alpha v \beta 3$  and  $\alpha v \beta 5$  or Arg-Gly-Asp (RGD)-containing peptides have been used. Integrin antagonist decreased colon cancer metastasis and angiogenesis and improved survival in a murine model (Reinmuth et al., 2003). Matrix metalloproteinases (MMPs) are essential for cell invasion. The tissue inhibitors of metalloproteinases (TIMPs), endogenous inhibitors, counterbalance the activity of MMPs. Application of AdTIMP-2 into pre-established tumors of colon cancer C51 in syngenic mice significantly reduced microvessel density (Li et al., 2001). VEGF has been shown to play a significant role in the course of angiogenesis. VEGF promotes endothelial cell proliferation, migration and survival, and in lymphangiogenesis promoted lymphatic tumor spread (Kawakami et al., 2003). Therefore, attacking one or more of the VEGF-mediated mechanisms may be promising in the treatment of colorectal neoplasms. Neutralizing anti-VEGF antibodies, blocking antibodies against the VEGFR-2 or selective inhibitors of the VEGFR-2 tyrosine kinase and VEGF pathway inhibitors have been applied, and resulted in a decrease in the growth and metastasis of colon cancer (Guba et al., 2004).

Apart from exogenous inhibitors, endogenous angiogenesis inhibitors can counter the balance between angiogenesis and anti-angiogenesis. Endostatin was an endogenous angiogenesis inhibitor, as an internal fragment of a matrix protein, collagen XVIII. It highly specifically inhibited proliferation and/or migration of endothelial cell (O'Reilly et al., 1997). Endostatin has a strong inhibitory effect both on tumor growth and metastases of human colon cancer through inhibiting tumor angiogenesis, and also exhibits a direct antitumor effect in colon cancer by inducing apoptosis (Zhang et al., 2002; Dkhissi et al., 2003). Combined with angiostatin, growth inhibition was observed in mice treated with a 1:1 proportion of angiostatin and endostatin genes, and the order of both genes transferred had a profound inhibitory effect on tumor growth (Uesato et al., 2004).

Recently, Watanabe and colleagues discovered a novel angiogenesis inhibitor, vasohibin, an endothelium-derived negative feedback regulator of angiogenesis. It inhibited migration, proliferation, and network formation

by ECs as well as angiogenesis *in vivo*, and served as an intrinsic and highly specific feedback inhibitor of activated endothelial cells engaged in the process of angiogenesis (Watanabe et al., 2004). A later study found that the expression of vasohibin in endothelial cells is regulated either positively or negatively by certain factors at the transcriptional level (Shimizu et al., 2005). These results suggest that exogenous vasohibin gene transfection or vasohibin protein administration might be powerful tools for the treatment of tumor angiogenesis.

#### Apoptosis induction

A balance between cell birth and cell death is necessary to sustain the colonic epithelium. Apoptosis, or programmed cell death, plays an important role in maintaining tissue homeostasis and permitting the controlled deletion of potentially harmful cells within the adult organism. Since most tumor cells have presumably disabled apoptosis to reach a malignant status, apoptosis induction represents one of the most obvious methods for cancer treatment.

At the molecular level, a number of genes, molecules or signals are often changed in colon cancer and some of these are key regulators of apoptosis, such as p53 mutant, IAP, and COX2. Direct targeting of the apoptotic pathway offers a novel strategy for overcoming apoptosis resistance. Tumor suppressor p53 gene, which is the most commonly mutated gene in human cancers, plays an important role in the apoptotic pathway. Re-expression with wild-type p53 in a TP53-mutated colon-cancer cell line in a murine model can cause growth inhibition and a doubling of survival times. Furthermore, the transfer of p53 genes into tumor cells lacking functional p53 enhanced the radio-responsiveness and the anti-tumor effect of common chemotherapeutic agent by promoting p53-dependent apoptosis (Magrini et al., 2002; Cook et al., 2004). Survivin is a member of the inhibitor of apoptosis (IAP) gene family, and is over-expressed in 53% to 64% of colorectal cancers (Sarela et al., 2001), which is correlated with carcinogenesis, angiogenesis, aggressive phenotype, and overall poor survival (Gianani et al., 2001; Kawasaki et al., 2001). Inhibition of surviving expression and function induced apoptosis and produced a phenotype of aberrant mitotic progression, characterized by supernumerary centrosomes, formation of multipolar mitotic spindles, failure of cytokinesis, and generation of multinucleated cells (Grossman et al., 2001). Our recent studies revealed that transduction of colon cancer cells with rAAV-Sur-Mut (Cys84Ala) inhibited cell proliferation and induced apoptosis and mitotic catastrophe *in vitro*. rAAV-SurA-Mut (Cys84Ala) sensitized colon cancer cells to chemotherapeutic drugs. Intratumoral injection of rAAV-Sur-Mut (Cys84Ala) significantly induced apoptosis and mitotic catastrophe and inhibited angiogenesis and tumor growth in a colon cancer xenograft model *in vivo*. More

importantly, rAAV-Sur-Mut (Cys84Ala) expression strongly enhanced the antitumor activity of 5-Fluorouracil (5-FU), resulting in the regression of established tumors (Tu et al., 2005).

Cyclooxygenase-2 (COX2) is an inducible isoenzyme of cyclooxygenase, undetectable in normal colonic mucosa and upregulated in 40% of adenomas and 85% of CRC (Eberhart et al., 1994). In the process of colorectal tumorigenesis, COX2 plays a key role in the various steps involved. COX-2 inhibitors can decrease the number and size of polyps and may prevent the progression from adenomatous polyp to invasive carcinoma, so that celecoxib was approved by the FDA for the treatment of polyps in patients with FAP (Steinbach et al., 2000). The COX-2-selective inhibitor NS398 also induces the release of cytochrome c from mitochondria and the elevation of caspase 9, caspase 3 and PARP enzymatic activities (Li et al., 2001), which contribute to apoptosis in colon cancer cell *in vitro* and inhibition of the growth of CRC cell lines *in vivo* (Williams et al., 2000).

Direct targeting of the death receptor can activate the apoptotic machinery and kill cancer cells. Death receptor pathways often remain intact in cancer. TNF- $\alpha$  and FasL are effective inducers of apoptosis, but they have very severe side-effects. Attempts to use TNF and FasL have been thwarted by induction of NF $\kappa$ B mediated inflammation and fulminant hepatic failure respectively, precluding their systemic use. In contrast, the family of TRAIL proteins and receptors has been studied by a considerable number of laboratories. Tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL) is a member of the TNF family of cytokines, which can induce apoptotic cell death in a variety of tumor cells by engaging the death receptors DR4 and DR5, while sparing most normal cells (Shi et al., 2003, 2005). During colorectal carcinogenesis, there is a marked increase in sensitivity to TRAIL-induced apoptosis associated with progression from benign to malignant tumor (Hague et al., 2005). The outgrowth of human colorectal tumors grown in mice was completely blocked by transduction with AAV.TRAIL *in vitro*, while *in vivo* transduction significantly inhibited the growth of established tumors (Mohr et al., 2004). The administration of soluble recombinant TRAIL by vein infusion also resulted in the elimination of metastatic colon cancer in the liver (Sova et al., 2004).

#### Perspective

Therapy for colon cancer has undergone considerable modifications in recent years. The protocol centered on fluorouracil has been joined by new drugs, including capecitabine, irinotecan, oxaliplatin, and targeted agents such as bevacizumab and cetuximab. Several new drugs are presently undergoing preclinical or clinical studies. However, the five-year survival rate from colon cancer has remained fairly static; standard therapy, however, remains non-specific; cytotoxic drugs

that are effective only in some patients, yet cause side-effects in most. Targeting of angiogenesis leads to minimal side effects as normal vessels are quiescent since the target is endothelial cells which are normal and genetically stable and the target of anti-angiogenic inhibitors, such as kallistatin, angiostatin and vasostatin, was activated endothelial cell. Anti-angiogenic inhibitors for cancer therapy with AAV-mediated provide a new strategy to span cancer patient's life (Xu et al., 2003).

Given the clinical and pathological heterogeneity of colorectal cancer, the optimal strategies for adjuvant therapy should be individualized. With the development of analysis and definition of prognostic and predictive markers, in future, therapeutic strategies should be constructed individually for each patient based on the molecular taxonomy of tumors, which would be lower toxicity, higher therapeutic index, and a weaker tendency to induce resistant phenotypes in tumor cells.

---

*Acknowledgements:* We thank Dr David Wilmshurst for the critical reading of this manuscript and Mrs Sue Xu for her assistance of manuscript preparation.

---

## References

- Brooks P., Clark R. and Cheresh D. (1994). Requirement of vascular integrin  $\alpha\text{V}\beta\text{3}$  for angiogenesis. *Science* 264, 569-571.
- Campos F., Logullo Waitzberg A., Kiss D., Waitzberg D., Habr-Gama A. and Gama-Rodrigues J. (2005). Diet and colorectal cancer: current evidence for etiology and prevention. *Nutr. Hosp.* 20, 18-25.
- Chu X., Chen L., Zang J., Wang J., Zhang Q. and Geng H. (2005) Effect of bone marrow-derived monocytes transfected with RNA of mouse colon carcinoma on specific antitumor immunity. *World J. Gastroenterol.* 11, 760-763.
- Cook T., Wang Z., Alber S., Liu K., Watkins S., Vodovotz Y., Billiar T. and Blumberg D. (2004). Nitric oxide and ionizing radiation synergistically promote apoptosis and growth inhibition of cancer by activating p53. *Cancer Res.* 64, 8015-80212.
- Dachs G., Tupper J. and Tozer G. (2005) From bench to bedside for gene-directed enzyme prodrug therapy of cancer. *Anticancer Drugs* 16, 349-359.
- DeVita V., Hellman S. and Rosenberg S. (2001). *Cancer Principles & Practice of Oncology*. 6th edn.
- Dkhissi F., Lu H., Soria C., Opolon P., Griscelli F., Liu H., Khattar P., Mishal Z., Perricaudet M. and Li H. (2003). Endostatin exhibits a direct antitumor effect in addition to its antiangiogenic activity in colon cancer cells. *Hum. Gene Ther.* 14, 997-1008.
- Eberhart C., Coffey R., Radhika A., Giardiello F., Ferrenbach S. and DuBois R. (1994). Upregulation of cyclooxygenase 2 gene expression in human colorectal adenomas and adenocarcinomas. *Gastroenterology* 107, 1183-1188.
- Fearon E. and Vogelstein B (1990). A genetic model for colorectal tumorigenesis. *Cell* 61,759-767.
- Friedlander M., Theesfeld C., Sugita M., Fruttiger M., Thomas M., Chang S. and Cheresh D. (1996). Involvement of integrins  $\alpha\text{V}\beta\text{3}$  and  $\alpha\text{V}\beta\text{5}$  in ocular neovascular diseases. *Proc. Natl. Acad. Sci. USA* 93, 9764-9769.
- Gianani R., Jarboe E., Orlicky D., Frost M., Bobak J., Lehner R. and Shroyer K. (2001). Expression of survivin in normal, hyperplastic and neoplastic colonic mucosa *Hum. Pathol.* 32,119-125
- Gonciarz M., Pierzchalski P., Lorens K., Pawlik W. and Petelenz M. (2004). Genetic aspects of colorectal carcinogenesis. *Wiad Lek.* 57, 74-79.
- Grossman D., Kim P.J., Blanc-Brude O.P., Brash D.E., Tognin S., Marchisio P.C. and Altieri D.C. (2001). Transgenic expression of survivin in keratocytes counteracts UVB-induced apoptosis and cooperates with loss of p53. *J. Clin. Invest.* 108, 991-999.
- Guanti G. and Bukvic N. (2000). Hereditary colorectal cancer syndromes. *Acta Chir. Iugosl.* 47, 23-25.
- Guba M., Seeliger H., Kleespies A., Jauch K. and Bruns C. (2004). Vascular endothelial growth factor in colorectal cancer. *Int. J. Colorectal Dis.* 19, 510-517
- Hague A., Hicks D., Hasan F., Smartt H., Cohen G., Paraskeva C. and MacFarlane M. (2005). Increased sensitivity to TRAIL-induced apoptosis occurs during the adenoma to carcinoma transition of colorectal carcinogenesis. *Br. J. Cancer* 92,736-742
- Holliger P., Manzke O., Span M., Hawkins R., Fleischmann B., Qinghua L., Wolf J., Diehl V., Cochet O., Winter G. and Bohlen H. (1999). Carcinoembryonic antigen (CEA)-specific T-cell activation in colon carcinoma induced by anti-CD3 x anti-CEA bispecific diabodies and B7 x anti-CEA bispecific fusion proteins. *Cancer Res.* 59, 2909-2916
- Jain R. (2005). Normalization of tumor vasculature: an emerging concept in antiangiogenic therapy. *Science* 307, 58-62.
- Kawakami M., Furuhashi T., Kimura Y., Yamaguchi K., Hata F., Sasaki K. and Hirata K. (2003). Quantification of vascular endothelial growth factor-C and its receptor-3 messenger RNA with realtime quantitative polymerase chain reaction as a predictor of lymph node metastasis in human colorectal cancer. *Surgery* 133, 300-308.
- Kawasaki H., Toyoda M., Shinohara H., Okuda J., Watanabe I., Yamamoto T., Tanaka K., Tenjo T. and Tanigawa N. (2001). Expression of survivin correlates with apoptosis, proliferation, and angiogenesis during human colorectal tumorigenesis. *Cancer* 91, 2026-2032
- Li H., Lindenmeyer F., Grenet C., Opolon P., Menashi S., Soria C., Yeh P., Perricaudet M. and Lu H. (2001). AdTIMP-2 Inhibits tumor growth, angiogenesis, and metastasis, and prolongs survival in mice. *Hum. Gene Ther.* 12, 515-526.
- Li M., Wu X. and Xu X. (2001). Induction of apoptosis in colon cancer cells by cyclooxygenase-2 inhibitor NS398 through a cytochrome c-dependent pathway. *Clin. Cancer Res.* 7, 1010-1016.
- Lipinski K., Djeha A., Ismail T., Mountain A., Young L. and Wrighton C. (2001). High-level, beta-catenin/TCF-dependent transgene expression in secondary colorectal cancer tissue. *Mol. Ther.* 4, 365-371.
- Longley D. and Johnston P. (2005). Molecular mechanisms of drug resistance. *J. Pathol.* 205, 275-292.
- Mack M., Riethmuller G. and Kufer P. (1995). A small bispecific antibody construct expressed as a functional single-chain molecule with high tumor cell cytotoxicity. *Proc. Natl. Acad. Sci. USA* 92, 7021-7025
- Magrini R., Bhone M., Hanski M., Notter M., Scherubl H., Boland C., Zeitz M. and Hanski C. (2002). Cellular effects of CPT-11 on colon carcinoma cells: dependence on p53 and hMLH1 status. *Int. J. Cancer* 101, 23-31.
- Mohr A., Henderson G., Dudus L., Herr I., Kuerschner T., Debatin K., Weiher H., Fisher K. and Zwacka R. (2004). AAV-encoded expression of TRAIL in experimental human colorectal cancer leads to tumor regression. *Gene Ther.* 11, 534-543.

- Narayan S. and Roy D. (2003). Role of APC and DNA mismatch repair genes in the development of colorectal cancers. *Mol. Cancer* 2, 41-55.
- Okaji Y., Tsuno N., Kitayama J., Saito S., Takahashi T., Kawai K., Yazawa K., Asakage M., Hori N., Watanabe T., Shibata Y., Takahashi K. and Nagawa H. (2004). Vaccination with autologous endothelium inhibits angiogenesis and metastasis of colon cancer through autoimmunity. *Cancer Sci.* 95, 85-90.
- Oosterhoff D., Overmeer R., de Graaf M., van der Meulen I., Giaccone G., van Beusechem V., Haisma H., Pinedo H. and Gerritsen W. (2005). Adenoviral vector-mediated expression of a gene encoding secreted, EpCAM-targeted carboxylesterase-2 sensitises colon cancer spheroids to CPT-11. *Br. J. Cancer* 14, 92, 882-887.
- O'Reilly M., Boehm T., Shing Y., Fukai N., Vasios G., Lane W. Flynn E., Birkhead J., Olsen B. and Folkman J. (1997). Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. *Cell* 88, 277-285.
- Parkin D. (2001). Global cancer statistics in the year 2000. *Lancet Oncol.* 2, 533-543.
- Parmiani G., Rodolfo M. and Melani C. (2000). Immunological gene therapy with ex vivo gene-modified tumor cells: a critique and a reappraisal. *Hum. Gene Ther.* 11, 1269-1275.
- Penuelas S., Noe V. and Ciudad C. (2005). Modulation of IMPDH2, survivin, topoisomerase I and vimentin increases sensitivity to methotrexate in HT29 human colon cancer cells. *FEBS J.* 272, 696-710.
- Reinmuth N., Liu W., Ahmad S., Fan F., Stoeltzing O., Parikh A., Bucana C., Gallick G., Nickols M., Westlin W. and Ellis L. (2003). Alphavbeta3 integrin antagonist S247 decreases colon cancer metastasis and angiogenesis and improves survival in mice. *Cancer Res.* 63, 2079-2087.
- Riethmuller G., Holz E., Schlimok G., Schmiegel W., Raab R., Hoffken K., Gruber R., Funke I., Pichlmaier H., Hirche H., Buggisch P., Witte J. and Pichlmayr R. (1998). Monoclonal antibody therapy for resected Dukes' C colorectal cancer: 7-year outcome of a multicenter randomized trial. *J. Clin. Oncol.* 16, 1788-1794.
- Riganti C., Miraglia E., Viarisio D., Costamagna C., Pescarmona G., Ghigo D. and Bosia A. (2005). Nitric oxide reverts the resistance to doxorubicin in human colon cancer cells by inhibiting the drug efflux. *Cancer Res.* 65, 516-525.
- Saha A., Chatterjee S., Foon K., Primus F., Sreedharan S., Mohanty K. and Bhattacharya-Chatterjee M. (2004). Dendritic cells pulsed with an anti-idiotypic antibody mimicking carcinoembryonic antigen (CEA) can reverse immunological tolerance to CEA and induce antitumor immunity in CEA transgenic mice. *Cancer Res.* 64, 4995-5003.
- Sarela A., Scott N., Ramsdale J., Markham A. and Guillou P. (2001). Immunohistochemical detection of the anti-apoptosis protein, survivin, predicts survival after curative resection of stage II colorectal carcinomas. *Ann. Surg. Oncol.* 8, 305-310.
- Schwartzberg L. (2001). Clinical experience with edrecolomab: a monoclonal antibody therapy for colorectal carcinoma. *Crit. Rev. Oncol. Hematol.*, 40, 17-24.
- Shi J., Zheng D., Man K, Fan S. and Xu RA (2003). TRAIL: a potential agent for cancer therapy. *Curr. Mol. Med.* 3, 727-736.
- Shi J., Zheng D., Liu Y, Sham MH, Tam P., Frazaneh F. and Xu RA (2005). Over-expression of soluble TRAIL induces apoptosis in human lung adenocarcinoma and inhibits growth of tumor xenografts in nude mice. *Cancer Res.* 65, 1687-1692.
- Shimizu K., Watanabe K., Yamashita H., Abe M., Yoshimatsu H., Ohta H., Sonoda H. and Sato Y. (2005). Gene regulation of a novel angiogenesis inhibitor, vasohibin, in endothelial cells. *Biochem. Biophys. Res. Commun.*, 327, 700-706
- Sova P., Ren X., Ni S., Bernt K., Mi J., Kiviat N. and Lieber A. (2004). A tumor-targeted and conditionally replicating oncolytic adenovirus vector expressing TRAIL for treatment of liver metastases. *Mol. Ther.* 9, 496-509-
- Steinbach G., Lynch P., Phillips R., Wallace M., Hawk E., Gordon G., Wakabayashi N., Saunders B., Shen Y., Fujimura T., Su L. and Levin B. (2000). The effect of celecoxib in familial adenomatous polyposis. *N. Engl. J. Med.*, 342, 1946-1952.
- Teng M., Kershaw M., Moeller M., Smyth M. and Darcy P. (2004). Immunotherapy of cancer using systemically delivered gene-modified human T lymphocytes. *Hum Gene Ther.* 15,699-708
- Tu S., Cui J., Liston P., Huajiang X., Xu R., Lin M., Zhu Y., Zou B., Ng S., Jiang S., Xia H., Wong W., Chan A., Yuen M., Lam S., Kung H. and Wong B. (2005). Gene therapy for colon cancer by adeno-associated viral Vector-mediated transfer of survivin Cys84Ala mutant. *Gastroenterology* 128, 361-375.
- Uesato M., Gunji Y., Tomonaga T., Miyazaki S., Shiratori T., Matsubara H., Kouzu T., Shimada H., Nomura F. and Ochiai T. (2004). Synergistic antitumor effect of antiangiogenic factor genes on colon 26 produced by low-voltage electroporation. *Cancer Gene Ther.* 11, 625-632.
- Uyl-de Groot C., Vermorken J., Hanna M., Verboom P., Groot M., Bonsel G., Meijer C. and Pinedo H. (2005). Immunotherapy with autologous tumor cell-BCG vaccine in patients with colon cancer: a prospective study of medical and economic benefits. *Vaccine* 23, 2379-2387.
- Watanabe K., Hasegawa Y., Yamashita H., Shimizu K., Ding Y., Abe M., Ohta H., Imagawa K., Hojo K., Maki H., Sonoda H. and Sato Y. (2004). Vasohibin as an endothelium-derived negative feedback regulator of angiogenesis. *J. Clin. Invest.* 114, 898-907.
- Williams C., Watson A., Sheng H., Helou R., Shao J. and DuBois R. (2000). Celecoxib prevents tumor growth in vivo without toxicity to normal gut: lack of correlation between in vitro and in vivo models. *Cancer Res.* 60, 6045-6051.
- Wils J., O'Dwyer P. and Labianca R. (2001). Adjuvant treatment of colorectal cancer at the turn of the century: European and US perspectives. *Ann. Oncol.* 12, 13-22.
- Xu R.A., Sun X., Tse L.Y., Li H., Chan P.C., Xu S., Xiao W., Kung H.F., Krissansen G.W. and Fan S.T. (2003) Long-term expression of angiostatin and suppression of liver metastatic cancer. *Hepatology* 37, 1451-1461
- Zhang G., Wang Y., Zhang M., Wang Q., Luo Y., Wang D. and Han C. (2002). Inhibition of growth and metastases of human colon cancer xenograft in nude mice by angiogenesis inhibitor endostatin A.i *Zheng* 21, 50-53.
- Zhu H., Guo W., Zhang L., Davis J., Teraishi F., Wu S., Cao X., Daniel J., Smythe W. and Fang B. (2005). Bcl-XL small interfering RNA suppresses the proliferation of 5-fluorouracil-resistant human colon cancer cells. *Mol. Cancer Ther.* 4, 451-456.