Review

Advancements of antisense oligonucleotides in treatment of breast cancer [1]

YANG Shuan-Ping, SONG San-Tai, SONG Hai-Feng[2,3]

Affiliated Hospital, [2]Department of Pharmacology, Institute of Radiation Medicine, Academy of Military Medical Sciences, Beijing 100850, China

KEY WORDS breast neoplasms; antisense oligonucleotides; oncogenes

ABSTRACT

Breast cancer is one kind of multi-gene related malignancy. Overexpression of some oncogenes such as *HER*-2 (c-*erbB*-2, *Neu*), *bcl*-2/*bcl*-xL, protein kinase A (PKA), and transferrin receptor gene (*TfR* gene), *etc* significantly affect the prognosis of breast cancer. It was shown that specific suppression of the overexpressed genes above resulted in the improvement of the therapy of breast cancer. Antisense interference, one of useful tools for inhibiting the overexpression of specific oncogenes, was involved in the therapy of breast cancer in recent years. Data indicated that antisense oligonucleotides (ON) could inhibit specially the expression of the target genes on mRNA or protein levels in most of cases; some ON candidates showed encouraging therapeutic effects *in vitro* and *in vivo* on breast cancer cell lines or xenografts. Furthermore, the combination use of the antisense ON and normal chemotherapeutic agents indicated synergistic antitumor effects, which was probably the best utilization of antisense ON in the treatment of breast cancer.

INTRODUCTION

The notion that gene expression could be modified through use of exogenous nucleic acids derives from studies by Paterson *et al*[1], who first used single-stranded DNA to inhibit translation of a complementary RNA in 1977. In the following year, Stephenson and Zamecnik[2] showed that a short (13nt) DNA oligonucleotide reverse complementary in sequence (antisense) to the Rous sarcoma virus could inhibit viral replication in culture. In 1983, the existence of naturally occurring antisense RNA, and their role in the regulation of gene expression was proven[3]. These observations were particularly important because they lent credibility to the belief that antisense was more than a laboratory phenomenon and encouraged belief in the hypothesis that reverse mentary could be used in living cells to manipulate gene expression. The developing of this technique directly resulted in appearance of a new class of drugs, antisense oligonucleotides (ON). According to the central dogma, genetic information was transmitted from DNA to mRNA by transcription, from mRNA to protein by translation. The intent effectively silence the gene of interest by preventing synthesis of the protein that it encodes is nonetheless attractive because mRNA is much more accessible and is efficient to be manipulated than DNA[4]. At present, a larger body of studies have focused on destabilizing mRNA directing at various targets that play a role in cancer[5,6], cardiovascular disease, viral disease, and inflammatory, accompany-

[1] Project supported by National Natural Science Foundation of China, No 30070895.
[3] Correspondence to Assoc Prof SONG Hai-Feng.
Phn 86-10-6693-0304. Fax 86-10-6821-4653.
E-mail song_hf@hotmail.com
Received 2002-01-26 Accepted 2002-12-05

ing with gene mutation and/or overexpression.

Most tumors accompany with gene mutation and/or overexpression, which result in the activation of oncogene. This is a very important process during the pathogenesis of the tumors. Breast carcinoma is the most common malignancy in women in western countries. Data from Eastern countries also indicated that the morbidity of breast cancer is still rising year by year. Almost all breast cancers were found to accompany with gene mutation and/or overexpression. Plenty of studies showed that there were definite relations between gene overexpression and pathogenesis, development and prognosis of breast cancer[7]. Presently, genes that are found to be in relation to breast cancer include oncogenes, eg, *HER*-2 (c-*erbB*-2, *Neu*), *bcl*-2, c-*myc*, and *ras*, *etc*; tumor suppressor genes eg, *p53*; estrogen receptor gene, progestogen receptor gene, estrogen regulation protein gene, growth factor receptor gene, and cyclin, *etc*. Antisense drugs have the characteristics of high selectivity and affinity to its targets, quickly taking effects, relatively definite mechanism, to be easy to evaluate the destination and less side effects, *etc*. These characteristics make antisense treatment an attractive strategy to selectively modulate the expression of genes involved in the pathogenesis of diseases. Breast cancer provides this class of new drug an ideal platform for evaluating the effects of antisense interference and for making break through. Not only can antisense suppress the overexpressed genes, but it is still of great value in studying the mechanisms of gene overexpression.

In this review, we focused on the recent advancements of antisense ON targeting at the genes that have definite relations with breast cancer. We will still concern some problems presently faced with.

SELECTION OF BREAST CANCER-RELATED GENES AS POTENTIAL TARGETS FOR ANTISENSE OLIGONUCLEOTIDES

HER*-2/c-*erbB*-2/*Neu The level of the M_r 185 000 *HER*-2 protein, encoded by the *HER*-2 oncogene, was elevated in approximately 30 % of cases involving human breast carcinoma[8]. Overexpression of *HER*-2 is widely considered as a poor prognosis following a tumor resection[9] and may be associated with increased resistance to cancer chemotherapy[10]. Herceptin, a humanized antibody generated against the *HER*-2 has been proven to be very effective in a phase II clinical trial involving patients with advanced stages of breast cancer, most of them expressing *HER*-2 at the highest levels[11]. This is the first therapeutic approach aimed at reducing the level of an oncogene product critically involved in breast cancer progression.

Bertram *et al*[12] selected two different regions of the *HER*-2 mRNA as potential antisense targets. These are the translation start region and the 3' translated region. The results showed that this design was very effective in reducing *HER*-2 expression in both SK-BR-3 and MCF-7 cell lines at a concentration as low as 2 μmol/L. Subsequently, Vaughn *et al*[13] evaluated the effects of another 15-mer phosphorothioate antisense oligonucleotides that included the start codon of *HER*-2 mRNA on *HER*-2 expression and cell cycle. Such antisense treatment also downregulates *HER*-2 mRNA and protein levels in a sequence-specific manner, and the *HER*-2 downregulation is accompanied by an accumulation of SK-BR-3 cell in G_1 phase of the cell cycle. Their research also indicated that the protein expression response to the PS antisense ON was biphasic, with a maximal downregulation achieved in the middle of the dosage range delivered by liposomes. Recently, the effect of the same sequence of antisense ON combined with several traditional chemotherapeutic agents, including doxorubicin hydrochloride, *cis*-platinum, and 5-fluorouracil on breast carcinoma cells growth were observed. The results showed a synergistic antitumor effect on BT-474 human breast carcinoma cells, one kind of high *HER*-2-expressed tumor cell line. However, in contrast to BT-474 breast carcinoma cells, there is no enhancement of these effects on low *HER*-2-expressed MCF-7 breast carcinoma cells with the same treatment[14]. This suggested that downregulation of *HER*-2 expression was able to increase the sensitivity of BT-474 cells to the cytotoxic effects of several traditional chemotherapeutic agents and this effect was directly in relation to the basic *HER*-2 expression level. The same reports also investigated the effect of this antisense ON in human tumor xenograft models *in vivo*. The results showed that systemic treatment with *HER*-2 antisense ON also significantly inhibited the growth of BT-474 xenografts in nude mice, the combination treatment using *HER*-2 antisense ON and doxorubicin resulted in an enhanced antitumor effect *in vivo*[14]. All these results indicate a potential clinical perspective of these antisense ON in the breast cancer therapy.

Bcl-2 and bcl-xL The *bcl*-2 family of proteins play major roles in regulating apoptosis and include both

anti- and pro-apoptotic members. Bcl-2 and bcl-xL are members of anti-apoptotic proteins. Expression of these proteins is significantly higher in primary breast cancers, where they might play a pivotal role in tumor initiation, progression, and resistance to chemotherapy and radiotherapy[15,16]. There is a more than 10-year history of antisense researches targeting at *bcl*-2 mRNA. G3139, which is being tested in clinical trials, is one of the few antisense ON targeting at the translational start region of the *bcl*-2 mRNA[17]. Chi *et al* observed the effects of G3139 on cell viability, bcl-2 protein expression, apoptosis of high bcl-2 protein expressing, estrogen receptor (ER) positive MCF-7 and low bcl-2 expressing, and ER negative MDA435/LCC6 human breast cancer cell lines. They found that treatment with G3139 *in vitro* caused a specific reduction of bcl-2 protein levels and increased apoptosis in both cell lines. These results suggested that the relative degree of downregulation of the bcl-2 protein was more important than the absolute reduction[18]. In the same work, combined treatment with G3139 and cytotoxic agents resulted in additive cytotoxicity in both cell lines. Furthermore, the initial clinical results of G3139 were still encouraging[19], 21 patients with bcl-2-positive relapsed NHL received a 14-d sc infusion of G3139, bcl-2 protein was reduced in 7 of 16 assessable patients, indicating its potential antitumor activity. oligonucleotide 4259 directed at breast cancer is the 2-*O*-methoxy-ethoxy antisense oligonucleotide targeting coding region of the *bcl*-xL mRNA. Treatment of MCF7 cells with oligonucleotide 4259 at a concentration of 600 nmol/L for 20 h decreased *bcl*-xL mRNA and protein levels by more than 80 % and 50 %, respectively and induced cell apoptosis. Moreover, the results that oligonucleotide 4259 resulted in similar effects in the breast carcinoma cell lines T-47D, ZR-75-1, and MDA-MB-231 were also observed in Simoes-Wust and his colleagues' experiments[20]. Oligonucleotide 4625 is another antisense ON targeting at a region of high homology shared by *bcl*-2 and *bcl*-xL mRNA. This oligonucleotide is also 2-MOE modified, 100 % complemented to *bcl*-2 mRNA and with 3 mismatches to *bcl*-xL mRNA. The results showed that 4625 treatment reduced *bcl*-2 and *bcl*-xL mRNA levels in a dose-dependent manner. In addition, the initial data of 4625 on breast carcinoma xenografts finished by Gautschi *et al* was also encouraging[21], oligonucleotide 4625 statistically significantly inhibited the growth of breast carcinoma xenografts by 51 %, it also reduced bcl-2 and bcl-xL protein levels and induced tumor cell apoptosis.

Protein kinase A (PKA) There are two types of PKA, PKAI, and PKAII, which share a C subunit but contain different regulatory R subunits, RI and RII, respectively. Through biochemical studies and gene cloning, four isoforms of the R subunits, RIα, RIβ, RIIα, and RIIβ have been identified. PKA plays a pivotal role in the control of cell growth and differentiation[22]. PKAI is involved in cell proliferation and neoplastic transformation, and is required for the G_1>S transition of the cell cycle. PKAI is overexpressed in the majority of human cancers, correlating with worse clinipathological features and prognosis in breast cancer patients[23]. Conversely, PKAII is preferentially expressed in normal tissues. Several studies investigated the RIα antisense targeting against NH_2-terminus 8-13 codons. Srivastava *et al*[24,25] sequentially evaluated the effects of this RIα antisense ON on tumor cell growth inhibition, mRNA and protein expression levels and apoptosis induction in different breast cancer cell lines. Their results indicated that this kind of antisense ON inhibited the growth of MDA-MB-231 breast cancer cells in a dose-dependent manner. The growth inhibitory effects correlated with a decrease in the RIα mRNA and protein levels. Similar growth inhibitory effects of this antisense ON were observed in MCF-7 breast cancer cell line, the growth inhibition was accompanied by an apoptosis induction, downregulation of RIα and upregulation of RIIβ protein expression. In the same study, they also found an interesting phenomenon that daily treatment of low doses of RIα antisense ON for 3 d which were tested to be ineffective doses for single use still significantly inhibited breast cancer cell growth. To evaluate the effect of blocking PKAI on MCF-10A cell sensitivity to taxanes, Ciardiello *et al*[26] treated these cells with taxol or taxotere in combination with the PKAI antisense ON. Their results indicated that treatment with this agent was able to overcome the effect of *HER*-2 overexpression on MCF-10A cell sensitivity to taxol and taxotere.

Transferrin receptor gene (*TfR* gene) Iron is required for the activity of ribonucleotide reductase, a key enzyme involved in DNA synthesis. TfR is the principal transport protein that mediates iron uptake into cells. TfR expression correlates with cellular proliferation and is found higher in rapidly dividing cells. The density of transferring receptors has also been correlated with the rate of DNA synthesis and metastatic potential of tumor cells[27,28]. Aggressive breast carci-

nomas that usually carry a poor prognosis had significantly higher TfR expression compared to grade I lesions with better prognosis[29]. Yang *et al* synthesized a 24-mer nucleotide complementary to the sequence corresponding to the translational initiation AUG codon of the TfR mRNA. They found that after exposure to antisense TfR-ON 1 μmol/L for 72 h, the number of live MCF-7, T47-D, and MDA-MB-231 tumor cells was significantly reduced. The IC_{50} (50 % inhibition of DNA synthesis) of TfR ON for the MCF-7, T47-D, and MDA-MB-231 cells were 0.5, 0.5, and 1.0 μmol/L, respectively, whereas the IC_{50} to normal breast cells was 30 μmol/L. Additionally, inhibitions of mRNA and protein synthesis induced by the same TfR antisense ON to these breast cancer cell lines were also observed by Yang and his coworkers[30].

V integrin gene Integrins are cell surface glycoprotein and consist of I heterodimers. At least 16 α and 8 β subunits have been described in mammals and these subunits can combine to form 22 different heterodimers, each with a specific recognition and binding affinity toward the various components of the extracellular matrix (ECM) milieu and cell adhesion molecules[31,32]. Integrins are not only implicated in cell-cell and cell-ECM interaction but have been shown to play a critical role in cell signaling, migration, differentiation, and tissue modeling[33]. There is considerable evidence for altered integrin levels in breast cancer cell lines. Levels of αV integrin protein were increased and could play a major role in breast carcinoma metastasis[34]. Based on the above data, Townsend *et al*[35] designed a 18-mer αV integrins antisense ON and tested the antiadhesive potential and expression-inhibiting effects of the αV integrin antisense ON on breast cancer cell line. They found that this antisense also significantly reduced αV mRNA transcription and protein expression in a dose- and time-dependent manner and promoted apoptosis of MDA-MB-231 cells .

Other targets It was recently reported that the mouse double minute 2 (*MDM*2) gene was amplified in breast cancer. Recently, Wang *et al*[36] selected MCF-7 cell line containing wild-type p53 and MDA-MB-468 cell line containing mutant p53 to evaluate the effects of MDM2 antisense treatment on p53 and p21 protein levels in both cell lines. They found that in MCF-7 cells, p53, and p21 protein levels were elevated as a result of reduced MDM2 expression. On the other hand, level of the p53 protein remained unchanged in MDA-MB-468 cells after treatment with MDM2 antisense ON. The same study also examined the effectiveness of the MDM2 antisense ON treatment *in vivo*. In nude mice bearing MCF-7 or MDA-MB-468 xenografts, MDM2 antisense dose-dependently inhibited the tumor growth. In both these models, synergistically or additive therapeutic effects of MDM2 inhibition were also observed when MDM2 antisense ON were combined with some other chemotherapeutic agents commonly used in the clinics such as irinotecan, 5-fluorouracil, and paclitaxel (Taxol). In addition, thymidylate synthase (TS) which was a key enzyme involved in the synthesis of DNA had been targeted for cancer chemotherapeutic agents[37]. Vascular endothelial growth factor (VEGF) and protein kinase Cα (PKCα) as candidate targets for breast cancer were also reported and need further investigations.

CLINICAL RESEARCH

The promising results of the use of antisense ON *in vitro* strongly stimulate the interests of the researchers to further investigate their effects *in vivo*, and consequently lead to development of several clinical trials. Antisense is rapidly moving from being a laboratory tool to becoming a full-fledged therapeutic strategy to treat various human diseases. At present, there are more than 20 clinical trials under way using antisense compounds directed at various targets that play roles in cancer[5,6], viral disease[38], and inflammatory disorders[39]. In July of 1998, the Food and Drug Administration (FDA) approved for marketing the first antisense-based therapeutic called fomivirsen which targets cytomegaloviral retinitis[40]. The investigations from Jansen and his colleagues[41] brought new hope for the idea of antisense, which showed that, besides the clinical benefit for patients with advanced melanoma, systemic treatment with antisense ON also results in the downregulation of the target protein within the target tissue. This finding will certainly stimulate the progress of antisense ON in clinical research in the treatment of breast cancer, since the results suggest that the principle of antisense works, not only with local treatment, as shown with fomivirsen[40], but also with systemic treatment with antisense ON. At present, the proof of clinical efficacy of antisense target at breast cancer is still missing. Though G3139 was undergoing clinical trails, this antisense compound was presently administered in non-Hodgkin's lymphoma[15]. Nevertheless, the initial encouraging antitumor effects of antisense ON target at *HER*-2 finished by Roh *et al*[14] suggested its

potential clinical value in the near future. The beauty and future potential of antisense ON in the treatment of breast cancer also depends on the design of multiple drugs based on our increasing knowledge of genes and their functions. We believe that as the deepening of the basic researches and accumulating of more basic data, the application of antisense ON in the treatment of breast cancer in clinics will be finally realized.

PROBLEMS AND PERSPECTIVES

Despite the short history of antisense research, numerous companies have already invested billions of dollars in exploring the potential of its use in treatments of various human diseases. Nevertheless, there is still long way to go before antisense drugs becoming one kind of mature and extensively used drugs. The use of antisense ON to fight against breast cancer also faced its own problems. First of all is the design of the antisense, not all antisense drugs complemented to the targeted mRNA are effective. In spite of the significant progress using advanced technology to predict mRNA secondary structure and phylogenesis analysis[42,43], the selection of the target sequences in designing effective antisense ON remains problematic. Second, the oligonucleotide must be taken in by the target cell, tissue or organ in a quantity sufficient to invoke a biological response, antisense needs efficient system for drug delivery. Third is the stability of the antisense oligonucleotide, presently, many researchers try to enhance the stability of the antisense *in vivo* through modification of base, ribose, and oligonucleotide skeleton. Fourth, diverse clinical outcomes of antisense were gotten in different laboratories. It is important to note that there is currently a major dichotomy between *in vitro* and *in vivo* studies of antisense effects. Some nonsequence-specific effects of antisense still could not be ruled out. In addition, expensive cost, dose-dependent side effects such as hypotension, complement activation, lymphoid hyperplasia, splenomegaly, and prolongation of thromboplastin time are problems of antisense presently faced with[44].

Like all other new developing technologies, antisense technology is still not a mature technology. More expectations for antisense ON are quite reasonable. Present initial experiments indicated that inhibition of most genes in relation to breast cancer by specific antisense ON could inhibit breast cancer cell growth and reduce the expression of mRNA and protein *in vitro* and *in vivo*. These results are encouraging and thus suggest that to select antisense ON as a common measure to manipulate the target gene is a quite effective method in those cells, tissues or organs accompany with gene overexpression. The understanding of antisense conception will be sublimated as the accumulation of more data and may finally result in some new findings. Immune stimulation is generally recognized as an undesirable side effect of certain antisense ON. Nevertheless, CpG oligonucleotides, which are designed to provide optimum immune stimulation, are promising anticancer drugs. G3139 that contains two CG dinucleotides and a TC at the 5' end described above was successfully used as an immunostimulatory CpG oligonucleotide in animal tumor models[45]. This new function of antisense should certainly benefit its antitumor effects and be sure to increase the complexity of antisense mechanism. It seems that the potential applications of antisense ON, this kind of nontoxic drug in the treatment of breast cancer in the near future maybe include following situations: single administration as a new kind of antitumor drugs; provocation of immune stimulation to enhance the anti-tumor effects, especially for those patients with immune system deficiency; combination with chemotherapeutic agents to produce synergistic antitumor effects, which may be the most probably utilization of antisense ON.

In conclusion, at present critical time, there are opportunities as well as dangers for antisense researches. As the deepening of the basic research and accumulation of the experimental data, antisense, this new class of drug will become mature. It now appears that antisense technology, which represents a new class of drugs, has nearly reached maturity and will have an important clinical role in the treatment of breast cancer[46].

ACKNOWLEDGMENT We thank for assistance and advices on English grammar revising by GUO Zhi-Hong and Sic L CHAN (Laboratory of Neurosciences, Gerontology Research Center, National Institute on Aging, 5600 Nathan Shock Drive, Baltimore, Maryland 21224, USA).

REFERENCES

1 Paterson BM, Roberts BE, Kuff EL. Structural gene identification and mapping by DNA-mRNA hybrid-arrested cell-free translation. Proc Natl Acad Sci USA 1977; 74: 4370-4.

2 Stephenson ML, Zamecnik PC. Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide.

Proc Natl Acad Sci USA 1978; 75: 285-8.
3 Simons RW, Kleckner N. Translational control of IS10 transposition. Cell 1983; 34: 683-91.
4 Liebhaber SA. mRNA stability and the control of gene expression. Nucliec Acids Symp Ser 1997; 36: 29-32,
5 Zhu YG, Zhuo GS, Chen ZZ, Chen XC. Cationic lipids enhanced cellular uptake and activity of bcl-2 antisense oligodeoxynucleotide G3139 in HL-60 cells. Acta Pharmacol Sin 2001; 22: 1007-12.
6 Yuan SJ, Tang ZM, Song HF, Zhu BZ. Combination of antisense oligodeoxynucleotides against C-raf and PKC-α mRNA enhancing inhibition on the proliferation of A549 cell *in vitro*. Chin J Pharmacol Toxicol 2000; 14: 136-9.
7 Walker RA, Jones JL, Chappell S, Walsh T, Shaw JA. Molecular pathology of breast cancer and its application to clinical management. Cancer Metastasis Rev 1997; 16: 5-27.
8 Menard S, Tagliabue E, Campiglio M, Pupa SM. Role of *HER*2 gene overexpression in breast carcinoma. J Cell Physiol 2000; 182: 150-62.
9 Kern JA, Schwartz DA, Nordberg JE, Weiner DB, Greene MI, Torney L, *et al*. P185neu expression in human lung adenocarcinomas predicts shortened survival. Cancer Res 1990; 50: 5184-7.
10 Pegram MD, Finn RS, Arzoo K, Beryt M, Pietras RJ, Slamon DJ. The effect of *HER*-2/neu overexpression on chemotherapeutic drug sensitivity in human breast and ovarian cancer cells. Oncogene 1997; 15: 537-47
11 Miles DW. Update on *HER*-2 as a target for cancer therapy: Herceptin in the clinical setting. Breast Cancer Res 2001; 3: 380-4.
12 Bertram J, Killian M, Brysch W, Schlingensiepen KH, Kneba M. Reduction of *erbB*2 gene product in mamma carcinoma cell lines by erbB2 mRNA-specific and tyrosine kinase consensus phosphorothioate antisense oligonucleotides. Biochem Biophys Res Commun 1994; 200: 661-7.
13 Vaughn JP, Iglehart JD, Demirdji S, Davis P, Babiss LE, Caruthers MH, *et al.* Antisense DNA downregulation of the ERBB2 oncogene measured by a flow cytometric assay. Proc Natl Acad Sci USA 1995; 92: 8338-42.
14 Roh H, Hirose CB, Boswell CB, Pippin JA, Drebin JA. Synergistic anti-tumor effects of *HER*2/neu antisense oligodeoxynucleotides and conventional chemotherapeutic agents. Surgery 1999; 126: 413-21.
15 Olopade OI, Adeyanju MO, Safa AR, Hagos F, Mick R, Thompson CB, *et al*. Overexpression of bcl-x protein in primary breast cancer is associated with high tumor grade and nodal metastases. Cancer J Sci Am 1997; 3: 230-7.
16 Reed JC: Prevention of apoptosis as a mechanism of drug resistance. Hem Onc Clinics North Am 1995; 9: 451-73.
17 Waters JS, Webb A, Cunningham D, Clarke PA, Raynaud F, di Stefano F, *et al*. Phase I clinical and pharmacokinetic study of bcl-2 antisense oligonucleotide therapy in patients with non-Hodgkin's lymphoma. J Clin Oncol 2000; 18: 1812-23.
18 Chi KC, Wallis AE, Lee CH, de Menezes DL, Sartor J, Dragowska WH, *et al*. Effects of bcl-2 modulation with G3139 antisense oligonucleotide on human breast cancer cells are independent of inherent bcl-2 protein expression. Breast Cancer Res Treat 2000; 63: 199-212.
19 Jansen B, Wacheck V, Heere-Ress E, Schlagbauer-Wadl H, Hollenstein U, Lucas T, *et al*. A phase I-II study with dacarbazine and BCL-2 antisense oligonucleotide G3139 (Genta) as a chemosensitizer in patients with advanced malignant melanoma (Abstract). Proc Am Asso Clin Oncol 1999; 18: 2049.
20 Simoes-Wust AP, Olie RA, Gautschi O, Leech SH, Haner R, Hall J, *et al*. Bcl-xl antisense treatment induces apoptosis in breast carcinoma cells. Int J Cancer 2000; 87: 582-90.
21 Gautschi O, Tschopp S, Olie RA, Leech SH, Simoes-Wust AP, Ziegler A, *et al*. Activity of a novel bcl-2/bcl-xL-bispecific antisense oligonucleotide against tumors of diverse histologic origins. J Natl Cancer Inst 2001; 93: 463-71.
22 Cho-Chung YS, Clair T. The regulatory subunit of cAMP-dependent protein kinase as a target for chemotherapy of cancer and other cellular dysfunctional-related diseases. Pharmacol Ther 1993; 60: 265-88.
23 Miller WR, Watson DM, Jack W, Chetty U, Elton RA. Tumour cyclic AMP binding proteins: an independent prognostic factor for disease recurrence and survival in breast cancer. Breast Cancer Res Treat 1993; 26: 89-94.
24 Srivastava RK, Srivastava AR, Park YG, Agrawal S, Cho-Chung YS. Antisense depletion of RIalpha subunit of protein kinase A induces apoptosis and growth arrest in human breast cancer cells. Breast Cancer Res Treat 1998; 49: 97-107.
25 Srivastava RK, Srivastava AR, Seth P, Agrawal S, Cho-Chung YS. Growth arrest and induction of apoptosis in breast cancer cells by antisense depletion of protein kinase A-RI alpha subunit: p53-independent mechanism of action. Mol Cell Biochem 1999; 195: 25-36.
26 Ciardiello F, Caputo R, Pomatico G, De Laurentiis M, de Placido S, Bianco AR, *et al*. Resistance to taxanes is induced by c-erbB-2 overexpression in human MCF-10A mammary epithelial cells and is blocked by combined treatment with an antisense oligonucleotide targeting type I protein kinase A. Int J Cancer 2000; 85: 710-5.
27 Neckers LM. Regulation of transferrin receptor expression and control of cell growth. Pathobiology 1991; 59: 11-8.
28 Cairo G, Pietrangelo A. Transferrin receptor gene expression during rat liver regeneration. Evidence for post-transcriptional regulation by iron regulatory factor B, a second iron-responsive element-binding protein. J Biol Chem 1994; 269: 6405-9.
29 Yang DC, Head JF, Wang F, Elliott RL. Transferrin receptor mRNA and ferritin H-chain mRNA expression are associated with prognostic indicators for breast cancer. Proc Am Assoc Cancer Res 1997; 38: 419-20.
30 Yang DC, Jiang XP, Elliott RL, Head JF. Inhibition of growth of human breast carcinoma cells by an antisense oligonucleotide targeted to the transferrin receptor gene. Anticancer Res 2001; 21: 1777-87.
31 Hynes RO. Integrins: a family of cell surface receptors. Cell 1987; 48: 549-54.
32 Green LJ, Mould AP, Humpries MJ. The integrin beta subunit. Int J Biochem Cell Biol 1998; 30: 179-84.

33 Meredith JE Jr, Wintitz S, McArthur Lewis J. The regulation of growth an intracellular signaling by integrins. Endocrine Rev 1996; 17: 207-20.
34 Meyer T, Marshall JF, Hart IR. Expression of αV integrins and vitronectin receptor identity in breast cancer cells. Br J Cancer 1998; 77: 530-6.
35 Townsend PA, Villanova I, Uhlmann E, Peyman A, Knolle J, Baron R, *et al*. An antisense oligonucleotide targeting the alphaV integrin gene inhibits adhesion and induces apoptosis in breast cancer cells. Eur J Cancer 2000; 36: 397-409.
36 Wang H, Nan L, Yu D, Agrawal S, Zhang R. Antisense anti-MDM2 oligonucleotides as a novel therapeutic approach to human breast cancer: *in vitro* and *in vivo* activities and mechanisms. Clin Cancer Res 2001; 7: 3613-24.
37 DeMoor JM, Vincent MD, Collins OM, Koropatnick J. Antisense nucleic acids targeted to the thymidylate synthase (TS) mRNA translation start site stimulate TS gene transcription. Exp Cell Res 1998; 243: 11-21.
38 Ho PT, Parkinson DR. Antisense oligonucleotides as therapeutics for malignant diseases. Semin Oncol 1997; 24: 187-202.
39 Robertson D. Crohn' s trial shows the pros of antisense. Nat Biotechnol 1997; 15: 209.
40 Anderson KP, Fox MC, Brown-Driver V, Martin MJ, Azad RF. Inhibition of human cytomegalovirus immediate-early gene expression by an antisense oligonucleotide complementary to immediate-early RNA. Antimicrob Agents Chemother 1996; 40: 2004-11.
41 Jansen B, Wacheck V, Heere-Ress E, Schlagbauer-Wadl H, Hoeller C, Lucas T, *et al*. Chemosensitization of malignant melanoma by BCL-2 antisense therapy. Lancet 2000; 356: 1728-33.
42 Song HF, Tang ZM, Yuan SJ, Zhu BZ. Application of secondary structure prediction in antisense drug design targeting protein kinase C-α mRNA and quantitative structure activity relationship analysis. Acta Pharmacol Sin 2000; 21: 80-6.
43 Song HF, Tang ZM, Yuan SJ, Zhu BZ. Activity prediction of antisense drugs designed against protein kinase C-α mRNA by QSAR equation. Chin J Pharmacol Toxicol 2000; 14: 401-4.
44 Galderisi U, Cascino A, Giordano A. Antisense oligonucleotides as therapeutic agents. J Cell Physiol 1999; 181: 251-7.
45 Weiner GJ. CpD DNA in cancer immunotherpy. Curr Top Microbiol Immunol 2000; 247: 157-70.
46 Yang SP, Song ST, Song HF, Chang XF. Antisense oligonucleotides, a new class of drug for breast cancer therapy. J Tumor Marker Oncology 2001; 16: 361-2.