Growth Inhibition of Human Colon Carcinoma Cells by Combinations of Anti-Epidermal Growth Factor-related Growth Factor Antisense Oligonucleotides

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ABSTRACT

GEO is a well-differentiated colon cancer cell line that coexpresses the epidermal growth factor-like growth factors CRIPTO (CR), amphiregulin (AR), and transforming growth factor-α (TGF-α). Antisense 20-mer phosphorothioate oligodeoxynucleotides (AS S-oligos) directed against CR, AR, and TGF-α mRNAs were equipotent in their ability to inhibit both the anchorage-dependent growth and the anchorage-independent growth (AIG) of GEO cells, with a 50% inhibitory concentration of about 5 μM in the AIG assay. A supraadditive effect was observed when a combination of S-oligos was used. For example, a combination of two different AS S-oligos (either AR + CR, or TGF-α + CR, or TGF-α + AR) at a concentration of 1 μM each (total concentration, 2 μM) resulted in 50% inhibition of GEO cells AIG, whereas the use of each AS S-Oligo at a 1 or 2 μM concentration resulted respectively in about 10 and 20% growth inhibition. A combination of the three AS S-oligos was even more effective, resulting in about 60% inhibition of GEO cells AIG at a concentration of 1 μM each (3 μM total concentration). The AS S-oligos were also able to inhibit specifically the expression of either AR, or TGF-α, or CR proteins in GEO cells, as assessed using immunocytochemistry or Western blot analysis. Finally, a supraadditive growth inhibitory effect of the AS S-oligos and an epidermal growth factor receptor-blocking antibody (monoclonal antibody 528) was observed. These data suggest that the use of a combination of AS S-oligos directed against different growth factors and antibodies directed against their receptors might result in an efficient inhibition of colon carcinoma cell growth.

INTRODUCTION

Growth factors and growth inhibitors are peptides that are involved in regulating normal cellular proliferation and differentiation, and that are important in initiating and/or maintaining cellular transformation (1). In fact, tumor cells generally exhibit a decreased requirement for exogenously supplied growth factors when compared to their normal counterparts. This relaxation in growth factor dependency may be due in part to the ability of transformed cells to synthesize peptide growth factors that can regulate their proliferation through autocrine, paracrine, and/or juxtacrine pathways (1). In this regard, normal and malignant human colon epithelial cells are able to synthesize several different growth factors (2). Among these, the EGF3-related peptides TGF-α, AR, and CR might play an important role in the pathogenesis of human colon carcinoma (3).

TGF-α is structurally and functionally related to EGF and acts exclusively through the EGF receptor (4). The mature 50-amino acid TGF-α peptide is proteolytically cleaved from a larger 160-amino acid transmembrane, glycosylated, and palmitoylated precursor that has biological activity (4). Approximately 50–90% of colon tumors express TGF-α, and a subpopulation of tumors contains higher levels of TGF-α protein than that of matched normal colon mucosa (5–9). Coexpression of TGF-α and EGF receptor has been detected in adult normal colon epithelium, colon villous adenomas and carcinomas, as well as in the majority of human colon carcinoma cell lines (10–13). These results suggest that an autocline loop involving TGF-α and EGF receptor is present in both normal proliferating colonic epithelial cells and colon carcinoma cells. Furthermore, TGF-α protein content in colon tumors does show a slight correlation with either tumor stage or histological grade, suggesting that overexpression of TGF-α might be associated with in vivo tumor progression (3).

AR is a recently discovered member of the EGF/TGF-α family of proteins (14). AR is a 78- or 84-amino acid, glycosylated, heparin-binding protein that is initially synthesized as a 252-amino acid transmembrane precursor (15). AR has an hy-
drophilic 43-amino acid NH₂-terminal extension that precedes the EGF-like domain and contains two putative nuclear-targeting sequences similar to those found in other DNA-binding proteins (15, 16). AR binds to the EGF receptor, generally with a lower affinity than EGF and TGF-α, and requires heparan sulfate-containing proteoglycans for EGF receptor binding (15, 17, 18). AR protein and mRNA have been found oversuppressed in approximately 50% of human primary colon carcinomas and in several colon carcinoma cell lines (19, 20). Furthermore, AR protein is preferentially expressed in well-differentiated human primary colon carcinomas when compared with poorly differentiated tumors (20).

The CR gene encodes a protein of 188 amino acids that contains a 37-amino acid region which shares a cysteine-rich motif in common with other members of the EGF family (21). A CR synthetic refolded peptide that corresponds to the EGF-like domain was able to stimulate the growth of both EGF receptor-positive and -negative human breast cancer cells (22). Furthermore, the CR synthetic peptide did not compete with EGF in binding to the EGF receptor, suggesting that CR can function as growth factor through an EGF receptor-independent pathway (22). High levels of CR mRNA and protein were found in a majority of human colon carcinoma cell lines and in 60–70% of human primary and metastatic colorectal tumors (19, 20). Since CR protein and mRNA are not usually expressed in normal colonic mucosa (20), they might represent a potential marker of colon tumorigenesis.

Coexpression of multiple growth factors of the EGF family occurs in colon carcinoma cells in vivo and in vitro (3). For example, the well-differentiated colon carcinoma cell line GEO expresses moderate levels of TGF-α, AR, and CR mRNAs and proteins (19, 20). Coexpression of CR and AR proteins was also observed in about 50% of the carcinomas and adenomas examined (20). Furthermore, it has been recently demonstrated that autoinduction and cross-induction among several growth factors of the EGF family occurs in colon carcinoma cells (23). This redundancy is not unique to colon cancer cells, since it also occurs in human breast cancer cells (24, 25).

Direct evidence that TGF-α, AR, and CR can function as autocrine growth factors in colon carcinoma cells has been recently established by using either neutralizing monoclonal antibodies or AS oligodeoxynucleotides (oligos) or AS mRNA retroviral expression vectors directed against these growth factors (26–30). In fact, the inhibition of either TGF-α, AR, or CR synthesis in colon carcinoma cells resulted in significant growth inhibition (27–30). Moreover, the growth of human colon carcinoma cells could be efficiently inhibited by using an EGF receptor-blocking antibody (26, 31), suggesting that an autocrine loop that involves the EGF receptor occurs in these cells.

The present study was designed to evaluate the possibility to develop experimental therapeutic approaches in human colon carcinoma by using combinations of AS oligos directed against various EGF-related growth factors and combinations of AS oligos and anti-EGF receptor-blocking antibodies.

MATERIALS AND METHODS

Cell Culture. GEO cells were kindly provided by Dr. M. Brattain (Baylor College of Medicine, Houston, TX). Cells were maintained in McCoy’s medium supplemented with 10% heat-inactivated fetal bovine serum, 20 mm HEPES (pH 7.4), penicillin (100 units/ml), streptomycin (100 μg/ml), Flow, Irvine, United Kingdom) in a humidified atmosphere of 95% air and 5% CO₂ at 37°C.

Materials. The S-oligos were synthesized on an Applied Biosystems model 381A oligonucleotide synthesizer. The S-oligos were subsequently purified on a Pharmacia Mono-Q fast-phase liquid chromatography ion exchange column using a linear gradient of 0.01–2.0 m tetrathyl ammonium bicarbonate (pH 8.0). Final purity of the S-oligos was confirmed by polyacrylamide gel electrophoresis. The purified S-oligos were then dialyzed against double-distilled water using M₃, 3500 cutoff dialysis tubing (Spectrum Medical Industries, Los Angeles, CA), lyophilized, and resuspended in sterile PBS. The sequences of the AS oligos were: TGF-α AS, 5’-TCCAGCCGAGGGGACCATTTTAC-3’; AR AS, 5’-GGGGCCTCTCATTCGCTTTC-3’; and CR AS, 5’-CTTGCAGTCTCATTTTATTTAC-3’. A mismatched random sequence was used as a negative missense S-oligo control.

The biological and biochemical characteristics of the EGF receptor-blocking MAb 528 have been previously described (32, 33).

ADG Assays. GEO cells (35 × 10³ cells/well) were seeded into 24-multiwell cluster dishes (Becton Dickinson, Milan, Italy) and treated every 24 h with the indicated concentrations of S-oligos for 3 days. After 4 days of growth, the cells were trypsinized and counted with a hemocytometer.

AIG Assays. GEO cells (5 × 10³ cells/well) were seeded in 0.3% Difco Bactoagar (Difco, Detroit, MI) supplemented with complete culture medium. This suspension was layered over 0.5 ml 0.8% agar-medium base layer in 24-multiwell cluster dishes (Becton Dickinson). The cells were treated 2 h and 5 days after seeding with the indicated concentrations of S-oligos. After 12 days, the colonies were stained with nitroblue tetrazolium, and colonies larger than 50 μm were counted with an Artex 880 colony counter (Artex Systems, Farmingdale, NY).

[³H]Thymidine Incorporation Assay. GEO cells (5 × 10³ cells/well) were plated in 96-multiwell cluster dishes (Becton Dickinson) and treated for 3 days with the indicated concentrations of S-oligos. The cells were then pulsed for 4 h with [³H]thymidine (1 μCi/ml) (Amersham Italia, Milan, Italy). Finally, the cells were precipitated with 10% trichloroacetic acid, dried, and solubilized with 0.1 m Tris-HCl (pH 8.0) containing 0.5% SDS. The lysates were quantitated for radioactivity by scintillation counting in Aquasol.

Immunocytochemistry. Immunocytochemical analysis of AR and CR expression in GEO cells grown in the presence or absence of S-oligos was performed as previously described (20). The antibodies used were the rabbit polyclonal AR-Ab1 anti-AR antibody, kindly provided by Dr. Gibbes R. Johnson (Food and Drug Administration, Bethesda, MD), and a rabbit polyclonal anti-CR antibody, kindly provided by Dr. W. J. Gullick (Imperial Cancer Research Fund, London, United Kingdom). The corresponding preimmune IgG was used as a negative control. The slides were evaluated by two investigators blinded to the treatment (E. d. A. and N. N.). Both intensity of staining and percentage of immunopositive cells were scored. Specific stain-
compared to the ADG assay. A supraadditive effect was observed when a combination of two different AS 5-oligos was used, because of its higher sensitivity when used. The supraadditive effect occurred for each concentration of AS 5-oligos and combination used, although it was more evident at low concentrations. For example, a combination of two different AS 5-oligos, either AR + CR, or TGF-α + CR, or TGF-α + AR, at a concentration of 1 μM each (total concentration 2 μM) resulted in 50% growth inhibition (Fig. 3).

RESULTS

GEO cells are a well-differentiated colon cancer cell line that coexpresses TGF-α, CR, and AR mRNAs and proteins (19, 20). GEO cells also express approximately 4 × 10³ EGF receptor sites/cell, and they are responsive to exogenous EGF and AR (31, 34). We have previously demonstrated that AS phosphorothioate oligodeoxynucleotides (AS S-oligos) directed against either AR and CR are able to significantly inhibit GEO cell growth and transformation (29, 30). We have also shown that infection of GEO cells with either a TGF-α or a CR AS mRNA retroviral expression vector results in a significant growth inhibition (27, 29). In this article, we have compared the efficacy of AS S-oligos directed against different EGF-related growth factors in inhibiting GEO cell growth.

AS S-oligos directed against either TGF-α, AR, or CR were equipotent in inhibiting the in vitro proliferation of GEO cells when used at equimolar concentrations. In particular, a concentration of 10 μM of either TGF-α, AR, or CR AS S-oligos inhibited by ~40% the ADG of GEO cells (Fig. 1B) and the [³H]thymidine incorporation (Fig. 1A) of GEO cells. The AIG of GEO cells was more significantly affected by AS treatment, since a 50% inhibitory concentration of approximately 5 μM was observed for the TGF-α, AR, or CR AS S-oligos (Fig. 2). No significant growth inhibition was observed when GEO cells were treated with concentrations of a missense S-oligo ranging from 1 to 10 μM (Figs. 1 and 2).

We chose the AIG assay to evaluate the efficacy of combinations of AS S-oligos because of its higher sensitivity when compared to the ADG assay. A supraadditive effect was observed when a combination of two different AS S-oligos was used. The supraadditive effect occurred for each concentration of AS S-oligos and combination used, although it was more evident at low concentrations. For example, a combination of two different AS S-oligos, either AR + CR, or TGF-α + CR, or TGF-α + AR, at a concentration of 1 μM each (total concentration 2 μM) resulted in 50% growth inhibition (Fig. 3).

Western Blot Analysis. Protein lysates (50 μg total protein/lane) were separated by SDS-PAGE (12% gel) and transferred to a nitrocellulose filter. The filter was incubated with a 1:500 dilution of the rabbit polyclonal anti-TGF-α antibody R9, kindly provided by Dr. B. Langton-Webster (Berlex Biosciences, Inc., Richmond CA). For protein visualization, the chemiluminescence ECL Western blotting kit (Amersham) was used according to the manufacturer’s specified procedure.

Table 1 Immunocytochemical analysis of AR and CR expression in GEO cells

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<thead>
<tr>
<th>Treatment</th>
<th>AR</th>
<th>CR</th>
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<tr>
<td>Control</td>
<td>+++</td>
<td>+++</td>
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<tr>
<td>AR AS-treated cells</td>
<td>++</td>
<td>n.d.</td>
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<tr>
<td>CR AS-treated cells</td>
<td>n.d.</td>
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<td>Missense-treated cells</td>
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* Cells were treated for 3 days with a 5 μM concentration of the indicated S-oligo.

† n.d., not determined.

![Fig. 1](image-url) A. effect of AS S-oligo treatment on the [³H]thymidine incorporation of GEO cells. GEO cells (5 × 10³ cells/well) were plated in 96-multiwell cluster dishes and cultured for 3 days in the absence (■) or presence of 10 μM of either missense (□), AR AS (▲), CR AS (●), or TGF-α AS (■) S-oligos for 3 days. The cells were then pulsed for 4 h with [³H]thymidine (1 μCi/ml). Finally, the cells were precipitated with 10% trichloroacetic acid, dried, and solubilized with 0.1 M Tris-HCl (pH 8.0) containing 0.5% SDS. The lysates were quantitated for radioactivity by scintillation counting in Aquasol. B. Effect of AS S-oligo treatment on the ADG of GEO cells. Cells (4 × 10⁴ cells/well) were seeded into 24-multiwell cluster dishes and treated every 24 h with a 10 μM concentration of either missense (□), AR AS (▲), CR AS (●), or TGF-α AS (■) S-oligos for 3 days. After 4 days of growth, the cells were trypsinized and counted with a hemocytometer. Control untreated cells (■), ~2 × 10⁴/well.
Fig. 2 Effect of AS 5-oligo treatment on the AIG of GEO cells. GEO cells (5 × 10^3 cells/well) were seeded in 0.3% Difco Bactoagar supplemented with complete culture medium. This suspension was layered over 0.5 ml 0.8% agar-medium base layer in 24-multiwell cluster dishes. The cells were treated 2 h and 5 days after seeding with 1 ( ), 2 ( ), 3 (•), 6 ( ), or 10 (□) μM concentration of the indicated 5-oligo. After 12 days, the colonies were stained with nitroblue tetrazolium, and colonies larger than 50 μm were counted with an Artek 880 colony counter. C. control untreated cells (U). 390 colonies/well.

whereas the use of each S-oligo at a 1 or 2 μM concentration resulted respectively in about 10 and 20% growth inhibition (Fig. 2). The supraadditive effect was even more evident when a combination of three different AS S-oligos was used. In fact, this combination resulted in about 60% growth inhibition at a concentration of each oligo of 1 μM (3 μM total concentration; Fig. 4), whereas the use of a single AS S-oligo at a 3 μM concentration resulted in about 30% growth inhibition (Fig. 2). Furthermore, the AIG of GEO cells was almost completely suppressed when cells were treated with 2 μM of each of the three AS S-oligos (Fig. 4).

The AS S-oligos were able to specifically inhibit the synthesis of TGF-α, AR, or CR proteins in GEO cells. Immunocytochemical analysis of GEO cells treated either with AR or CR AS S-oligos was performed using two specific polyclonal antibodies. Control untreated GEO cells showed a specific staining for both AR and CR. The staining was mostly cytoplasmic for CR, while cytoplasmic and occasionally nuclear staining for AR was observed, as previously described (Fig. 5, A and B). Treatment of GEO cells with AR AS or CR AS S-oligos at a concentration of 5 μM for 72 h resulted in a significant reduction respectively of AR and CR protein expression, whereas the missense S-oligo at the same concentration had no significant effect on the levels of both endogenous growth factors (Fig. 5 and Table 1). Western blot analysis also showed that GEO cells express four different forms of cell-associated TGF-α, with M, ranging from 18,000 to 30,000, that correspond to the pro- and meso-cell-associated forms of TGF-α (Fig. 6, Lane 1). Treatment of GEO cells with 5 μM TGF-α AS S-oligo for 72 h resulted in a significant reduction of the four species of endogenous TGF-α (Fig. 6, Lane 2), whereas no effects were observed when GEO cells were treated with the missense S-oligo at equimolar concentration (Fig. 6, Lane 3).

Finally, a supraadditive effect was also observed when a combination of AS S-oligos directed against either TGF-α, AR,
or CR and of an anti-EGF receptor-blocking MAb, MAb 528, was used. The MAb 528 was able to inhibit GEO cells AIG in a dose-dependent manner, as previously described (Fig. 7A; Ref. 31). In particular, the MAb 528 at a concentration of 1 μg/ml inhibited the AIG of GEO cells by 40%. A combination of MAb 528 (1 μg/ml) with 1 μM of either TGF-α, CR, or AR AS S-oligos resulted in 70% growth inhibition (Fig. 7B). The AIG of GEO cells was almost completely suppressed when a combination of MAB 528 and either one of the three AS S-oligos at a concentration of 2 μM was used, or when combinations of two AS S-oligos at a concentration of 1 μM each were used (Fig. 7B).

**DISCUSSION**

Colon cancer cells secrete and/or respond to several different peptide growth factors, such as TGF-α, TGF-β, AR, CR, insulin-like growth factor I, insulin-like growth factor II, gastrin, and platelet-derived growth factor, and express specific cell surface receptors for these peptides (2). An extensive body of evidence suggests that these growth factors may contribute through autocrine, paracrine, juxtacrine, and/or intracrine pathways to regulate tumor growth, angiogenesis, and metastasis (35). Therefore, peptide growth factors might represent suitable targets for experimental therapeutic approaches in colon tumors. In this context, there are several sites in growth factor-regulated pathways where therapeutic intervention might be possible: the synthesis and the secretion of the growth factor, the binding to the receptor, and the synthesis and/or the activation of the receptor and more generally of the proteins involved in the intracellular signal transduction.

AS oligonucleotides directed against proto-oncogenes and/or growth factors have been successfully used to inhibit the growth of several human cancer cell lines (36). For example, treatment of various melanoma and glioblastoma cell lines with basic fibroblast growth factor AS oligonucleotides inhibited their proliferation and colony formation in agar (37, 38). AS oligonucleotides directed against AR mRNA were able to inhibit the growth of nontransformed and ras- and c-neu-transformed human mammary epithelial cells (39, 40) as well as the growth of two gastric carcinoma cell lines (41). Furthermore, we have previously demonstrated that AS oligonucleotides directed against either AR or CR as well as a retroviral AS TGF-α expression vector are able to inhibit the growth of human colon carcinoma cells (27, 29, 30). Sizeland and Burgess (28) also showed that TGF-α AS oligonucleotides were efficient in inhibiting the growth of the LIM 12115 colon cancer cell line. This article is the first demonstration that equimolar concentrations of AS oligonucleotides directed against TGF-α, CR, and AR are equipotent in inhibiting the growth of a colon carcinoma cell line that coexpresses these three growth factors. We have also demonstrated that a supraadditive effect occurs when combinations of such oligonucleotides are used. This phenomenon might be due to a functional interaction between these EGF-related growth factors. In fact, it has been recently demonstrated that several EGF-related peptides are able to induce the expression of other members of the same family in colon carcinoma cells (23). Therefore, these data suggest that the proliferation of human colon carcinoma cells is sustained by a network of EGF-related peptides. This redundancy also suggests that each growth factor could play a different role in colon carcinoma cell proliferation, differentiation, and transformation. This might provide an explanation for the more significant growth inhibition observed when the expression of multiple growth factors of the same family is inhibited, as compared with the block of the expression of a single growth factor.

DNA phosphodiester oligonucleotides are rapidly degraded by nucleases that are usually present in the serum. Phosphorothioate DNA differs from natural phosphodiesters DNA only in the replacement of an oxide ligand on phosphorus by a sulfide (42). Phosphorothioate AS oligos have greater stability than normal oligos in biological fluids, since they are more resistant to serum nucleases. It has been observed that, if their concentration is not optimized, phosphorothioate oligonucleotides can be sequence-nonspecific inhibitors of protein synthesis, both in cell-free systems and intracellularly (36). In this context, the high efficacy of combinations of AS S-oligos that we have observed might allow the use of lower concentrations of S-oligos, as compared with the use of each S-oligo alone, to obtain a significant inhibition of tumor cell growth. This might reduce the possible occurrence of nonspecific effects on protein synthesis which could be potentially toxic for patients treated with phosphorothioate oligonucleotides.

Enhanced expression of the EGF receptor has been detected in the majority of glioblastomas and breast, lung, ovarian, colorectal, and renal carcinomas, suggesting that growth-regulating pathways that function through this receptor are involved in the pathogenesis of various types of human carcinomas (3). Several blocking anti-EGF receptor MAbs have been generated (32, 33, 43, 44). In particular, the MAb 528 is a mouse IgG2a
that binds to the EGF receptor with an affinity similar to that of EGF or TGF-α, competes with these ligands for receptor binding, and blocks EGF- or TGF-α-induced activation of EGF receptor tyrosine kinase (32). MAb 528 inhibits the in vitro and in vivo growth of numerous human cancer cell lines (45, 46). Our data demonstrate that a supraadditive effect occurs when combinations of AS-S-oligos directed against EGF-related growth factors and MAb 528 are used. This phenomenon might be due to several mechanisms. In fact, it has been demonstrated that CR functions as growth factor through activation of an EGF receptor-independent pathway (22). In this context, the growth inhibitory effect of CR AS might add to the blockade of the EGF receptor pathway. AR possesses two nuclear targeting sequences, and it is able to bind to double-strand DNA via a phosphoprotein (15, 47, 48). Immunoreactive AR has been detected in the nuclei of several breast and colon cancer cell lines and in human primary breast and colon carcinomas (20, 24, 34, 49). Furthermore, the AIG of ras- and neu-transformed human mammary epithelial cells is inhibited by AR AS S-oligos, but not by heparin, which is able to interfere with the binding of AR to the EGF receptor (40). Therefore, these data suggest that AR may function through an intracellular mechanism by direct interaction with nuclear receptors or alternatively through the intracellular binding to the EGF receptor, as it has been respectively demonstrated for basic fibroblast growth fac-

**Fig. 5** Immunocytochemical analysis of CR and AR expression in GEO cells. A and B, GEO cells were treated respectively with 5 μM missense or CR AS S-oligos for 72 h and reacted with anti-CR antibody. ×250. C and D, GEO cells were treated respectively with 5 μM missense or AR AS S-oligos for 72 h and reacted with anti-AR antibody. ×100.

**Fig. 6** Western blot analysis of TGF-α protein expression in GEO cells. Lane 1, control untreated GEO cells; Lane 2, cells treated for 3 days with 5 μM TGF-α AS S-oligo; and Lane 3, cells treated for 3 days with 5 μM missense S-oligo.
pared with the use of a single agent. In this context, colon carcinoma cells may synthesize other EGF-like growth factors, such as betacellulin and heparin binding-EGF (3), that also function through the EGF receptor.

The occurrence of a more than additive effect might be very important for future clinical applications. In fact, both AS S-oligos against EGF-like growth factors and the anti-EGF receptor-blocking antibody seems to have a cytostatic more than cytotoxic activity in vitro (50, 51). Furthermore, treatment with anti-EGF receptor MAb causes marked growth inhibition of human tumor xenografts in vivo, but does not consistently eliminate well-established xenografts (45, 52, 53). In this context, the supraadditive effect that occurs in vitro using combinations of AS S-oligo targeted against EGF-like growth factors and EGF receptor-blocking antibodies might result in an improvement of the in vivo anticancer efficacy of MAb 528. In this regard, we are currently planning in vivo studies to evaluate the efficacy of these combinations against human tumor xenografts in immunodeficient mice.

REFERENCES

of the epidermal growth factor family. Science (Washington DC), 243:


Becker, D., Meier, C. B., and Herlyn, M. Proliferation of human malignant melanomas is inhibited by antisense oligodeoxynucleotides targeted against basic fibroblast growth factor. EMBO J., 8: 3685–3691, 1989.


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