**erbB-2 antisense oligonucleotides inhibit the proliferation of breast carcinoma cells with erbB-2 oncogene amplification**

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**Summary**

Amplification and overexpression of the erbB-2 oncogene is an unfavourable prognostic marker in human breast cancer and occurs in approximately 25% of breast carcinomas. We used erbB-2 antisense oligonucleotides to inhibit the proliferation of human breast cancer cell lines. erbB-2 antisense oligonucleotides (20 \(\mu\)M) inhibited the growth and DNA synthesis of breast cancer cell lines with an amplified erbB-2 gene by up to 60%. Control complementary sense oligonucleotides did not inhibit cellular proliferation at the same concentration but showed inhibitory effects at higher concentrations. There was no specific effect of erbB-2 antisense oligonucleotides on breast cancer cell lines that had no amplification of erbB-2. erbB-2 antisense oligonucleotides reduced erbB-2 protein levels, measured by immunohistochemistry, in a dose-dependent manner. erbB-2 sense oligonucleotides did not decrease the levels of erbB-2 protein. These data indicate that erbB-2 antisense oligonucleotides induce a specific inhibition of erbB-2 protein expression and that erbB-2 gene overexpression is important for the proliferation of the breast cancer cells that have been selected for erbB-2 amplification.

The erbB-2 oncogene (also called neu and HER-2) codes for a 185 kDa transmembrane growth factor receptor with an intracellular tyrosine kinase catalytic domain (Schechter et al., 1985; Coussens et al., 1985; Yamamoto et al., 1986). The erbB-2 gene copy number is amplified in many adenocarcinomas and, in particular, erbB-2 is amplified or overexpressed in approximately 25% of human breast carcinoma samples (Maguire & Greene, 1989). Overexpression of erbB-2 has not been observed in normal adult human tissues (Press et al., 1990). Amplification or overexpression of erbB-2 in malignant breast tumours has been correlated with nodal metastases, early relapse and shortened survival (Slamon et al., 1987; Tandon et al., 1989; Wright et al., 1989; Paik et al., 1990). Moreover, expression of high levels of erbB-2 oncogene is sufficient to induce neoplastic transformation of some cell lines (DiFiore et al., 1987; Hudziak et al., 1987; Tarakhovsky et al., 1990). A role for erbB-2 in the aetiology of some breast carcinomas was further suggested by transgenic mouse experiments, in which animals expressing the erbB-2 gene under the control of a steroid-inducible promoter manifested a breast adenocarcinoma phenotype (Müller et al., 1988; Bouchard et al., 1989).

It has been shown that monoclonal antibodies against the erbB-2 oncoprotein applied to cells transformed by the rat neu oncogene cause them to revert to a non-transformed phenotype (Drebin et al., 1985; van Leeuwen et al., 1990), and these antibodies diminished the in vivo tumorigenicity of transplanted murine fibroblasts transformed by neu (Drebin et al., 1986, 1988). In addition, monoclonal antibodies to the human erbB-2 protein also inhibited the in vitro growth of the erbB-2-overexpressing human breast cancer cells SK-Br-3 by 56% (Hudziak et al., 1989). The antiproliferative effects of the erbB-2 antibodies are related to their ability to down-regulate the erbB-2 protein. While erbB-2 antibodies which reduce the levels of erbB-2 exhibit antiproliferative effects, monovalent F(ab) fragments (Drebin et al., 1986; Kumar et al., 1991; Sarup et al., 1991) or erbB-2 antibodies which do not down-regulate erbB-2 (Sarup et al., 1991; Tagliafuore et al., 1991) do not show growth-inhibitory effects.

Antisense oligonucleotides have been used for the specific study of gene expression. They also represent a new class of potential pharmacological agents for in vivo antiviral or antineoplastic therapy (Hélène, 1989; Tidd, 1990; Uhlmann & Peyman, 1990; Carter & Lemoine, 1993). Antisense oligonucleotides have been used to study the *in vitro* cellular effects of oncogenes and growth factors in cells from leu-kaemia (Holt et al., 1988; Wickstrom et al., 1988; Reed et al., 1990; Szczylak et al., 1991), lymphoma (McManaway et al., 1990) and solid cancers (Rosolen et al., 1990; Melani et al., 1991; Morrison, 1991; Saison-Behmoaras et al., 1991).

We report the effect of erbB-2 antisense oligonucleotides on cell proliferation and erbB-2 protein expression in breast cancer cells. The results show that the proliferation of human breast cancer cells—measured by cell growth and DNA synthesis—can be inhibited following treatment with antisense oligonucleotides and that this effect is specific for breast cancer cells which present erbB-2 amplification. In addition, expression of the erbB-2 oncogene product in breast cancer cells is reduced in a dose-dependent fashion by erbB-2 antisense, but not sense, oligonucleotides, indicating that overexpression of erbB-2 is important for the proliferation of those cells that have been selected for erbB-2 gene amplification.

**Materials and methods**

**Oligonucleotides**

Oligonucleotides were synthesised on a Milligen/Biosearch DNA Cyclone by means of phosphoramidite chemistry. The lyophilised product was dissolved in TE buffer (10 mM Tris Cl, 1 mM EDTA, pH 7.4) and stored at −20°C. We selected sequences from the 5’ end of the erbB-2 gene and performed a computer search in the GenBank database to obtain information about other possible sequence matches. For our experiments we chose a 21-nucleotide sequence directed against the 5’-translated end of erbB-2 that showed no sequence matches against the database, and a partially overlapping 15-mer that also included the erbB-2 initiation codon and had imperfect matches to human crystallin and keratin K7 genes. Their complementary sense sequences were used as controls. The oligonucleotide sequences are as follows: 1, 15-mer antisense, GGC GGC CAG CTC CAT; 2, 15-mer sense, ATG GAG CTG CGC GCC; 3, 21-mer antisense, GCG GCA CAA GGC CGC CAG CTC; 4, 21-mer sense, GAG CTG GCC GCC TTG TGC CGC. In some experiments, a 21-mer was synthesised with the same overall oligonucleotides content as 3, but with a random order (nonsense 21-mer).

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Cell lines
The breast cancer cell lines SK-BR-3, BT-474, MDA-MB-453, MDA-MB-361 and MDA-MB-468 were obtained from the American Tissue Culture Collection (Rockville, MD, USA) and maintained in Iscove's modified Eagle medium (IMEM) supplemented with 5% fetal calf serum (FCS) at 37°C in a 5% carbon dioxide incubator. MCF-7 cells are routinely passaged in our laboratory. SK-BR-3, BT-474, MDA-MB-453 and MDA-MB-361 cells have 4- to 8-fold amplification of erbB-2 (Kraus et al., 1987). erbB-2 protein expression of the cell lines was evaluated by immunostaining with an erbB-2 monoclonal antibody (no. 94, a gift from C.R. King, Molecular Oncology, Gaithersburg, MD, USA) and analysed on a fluorescence-activated cell sorter (FACStar Plus, Becton Dickinson). SK-BR-3, BT-474, MDA-MB-453 and MDA-MB-361 cells expressed elevated amounts of erbB-2 protein, while MCF-7 cells showed low levels, and MDA-MB-468 cells did not express detectable levels of the protein by FACS (data not shown).

Culture medium and oligonucleotide stability
To ensure that the oligonucleotides added to the cell cultures lasted for the duration of the assays, we tested the stability of oligonucleotides in different culture media. Serum-free medium consisted of IMEM (Biofluids) with the addition of fibronectin 2 mg l\(^{-1}\) (Collaborative Research), transferrin 5 mg l\(^{-1}\) (Sigma), 20 mM HEPES (Biofluids), trace elements (Biofluids), glutamine (Biofluids) and 10\(^{-2}\) M insulin (Biofluids). To assay the stability of oligonucleotides in media over time, oligonucleotides were added to IMEM containing 5% FCS or serum-free medium and incubated at 37°C. Samples of 0.4 ml of medium were aliquoted at specified times, phenol extracted, ethanol precipitated, and run on a 20% polyacrylamide gel. Seventy micrograms of a 20-mer oligonucleotide was incubated with 2.8 ml of serum-containing medium at 37°C. Twenty-five micrograms of a 21-mer was incubated with 2 ml of serum-free medium.

Uptake of fluorescein conjugated oligonucleotides
For oligonucleotide uptake experiments, aminolinker-derivatised oligonucleotides were synthesised on an Applied Biosystems 381A DNA synthesiser. Oligonucleotides were incubated with a fluorescein isothiocyanate (FITC) sodium carbonate/sodium bicarbonate – water – dimethylformamide (5:8:2) solution overnight in the dark. The reaction mixture was passed through a Sephadex G50 column to remove excess FITC, eluting with 20% ethanol in water. The fractions selected showed simultaneous absorption at 260 nm (DNA) and 500 nm (FITC).

To analyse cellular fluorescence, cells were plated in six-well plates at a concentration of 10\(^{6}\) cells per well and allowed to attach overnight. Medium was then changed to serum-free medium containing 50 \(\mu\)M fluorescein-labelled oligonucleotides, and incubated overnight. After washing the wells with phosphate-buffered saline (PBS) cells were detached with PBS–EDTA, and cellular fluorescence was quantitated using a fluorescence-activated cell sorter (FACStar Plus). To assess whether the fluorescence assessed by FACS was intracellular, we used fluorescence microscopy. Cells were plated in eight-well Lab-Tek chamber slides (Miles Labs, Naperville, IL, USA) at a concentration of 10,000 cells per well and allowed to attach overnight. Medium was then changed to serum-free medium containing 50 \(\mu\)M fluorescein-labelled oligonucleotides, and incubated overnight. The slide was washed, fixed with 2% formalin and covered with a coverslip. Samples were visualised by epi-illumination on a Zeiss Photomicroscope III.

Cell proliferation experiments
For cell growth assays, cells were plated in 24-well plates (Costar) in IMEM supplemented with 5% FCS to facilitate attachment to the culture dish. After 24 h, medium was changed to serum-free defined medium, with or without increasing concentrations of erbB-2 sense or antisense oligonucleotides. After 7 days, cells were detached from the wells with 5 mM EDTA in PBS and counted in a Coulter counter (Hialeah, FL, USA).

We also evaluated the effect of oligonucleotides on the incorporation of \(^{3}H\)thymidine into cellular DNA. SK-BR-3 cells were incubated for 4 h in a defined serum-free medium containing different concentrations of 15-mer erbB-2 antisense and sense oligonucleotides. The medium was then replaced with 250 \(\mu\)l of 10% FCS–IMEM supplemented with 10\(\mu\)Ci ml\(^{-1}\) \(^{3}H\)thymidine. After 3 h, cells were harvested, sonicated and treated with 10% trichloroacetic acid (TCA). TCA precipitates were collected on HA 0.45 \(\mu\)m filters (Millipore) and radioactivity was counted.

erbB-2 protein analysis
Immunohistochemical staining and quantitation of erbB-2 protein have been described previously (Bacus et al., 1990). In brief, exponentially growing SK-BR-3 cells were plated in four-chamber slides (Nunc, Napperville, IL, USA) at 0.5 \(\times\) 10\(^{6}\) cells per chamber. After 24 h, the medium was replaced with serum-free medium with or without erbB-2 oligonucleotides. After 6 days, cells were stained with the HER-2/new oncoprotein staining kit (Cell Analysis Systems, Elmhurst, IL, USA), using a monoclonal antibody against erbB-2 (Ab-2, Oncogene Science, Manhasset, NY, USA) at a concentration of 10 \(\mu\)g ml\(^{-1}\). Cells were counterstained with Feulgen DNA stain (Cell Analysis Systems). The CAS 200 Image Analyzer (Cell Analysis Systems), with its two solid-state image-sensing channels specifically matched to the two components of the stains used (blue stain for DNA and red stain for erbB-2 protein), was used to quantify average total erbB-2 protein per cell and the total cellular DNA. Sparsely growing cells were used for calibrating the cellular erbB-2 protein content. At least 300 cells were counted for each determination. The analyser sums the total intensity of protein staining and divides by the total number of cells counted to determine the staining index.

Results
Oligonucleotide stability and uptake
We first wanted to be sure that the oligonucleotides used to treat the cells were stable in culture media and were taken up by the cells. After exposure to serum-containing medium oligonucleotides underwent rapid exonucleolytic cleavage. At 2 h, a stepladder pattern of the synthetic DNA was apparent, and no oligonucleotide was detected in the gel after 10 h. On the other hand, oligonucleotides were not detectably degraded in serum-free medium, and the band was unchanged after up to 7 days of exposure. Therefore, we used serum-free medium for our experiments. The cell lines used in our study had equivalent growth in this medium as in medium containing 5% fetal calf serum.

Since we were going to study breast cancer cell lines which might incorporate oligonucleotides at different rates, we evaluated the uptake of fluorescein-conjugated oligonucleotides using a fluorescence-activated cell sorter. The mean cellular fluorescence of the different cell lines was similar after exposure to 50 \(\mu\)M FITC-labelled oligonucleotide, and did not correlate with the presence of erbB-2 gene amplification or with the cell size (Table I). We used fluorescence microscopy to study the pattern of cellular staining that resulted in our experiments with labelled oligonucleotides. After exposure cells to FITC-labelled oligonucleotides, the location of the fluorescent label was predominantly intracellular.

Inhibition of cell proliferation
The effect of erbB-2 antisense oligonucleotides on breast cancer cell growth was tested by cell proliferation assays,
including both cell growth and DNA synthesis assays. The complementary sense oligonucleotides were used as controls in all experiments. We observed that the addition of erbB-2 antisense oligonucleotides to cell lines that have an amplified erbB-2 oncogene resulted in a dose-dependent inhibition of cell growth. Treatment of SK-BR-3 cells for 7 days with 20 \( \mu \)M 21-mer erbB-2 antisense oligonucleotide resulted in a 57% cell growth inhibition when compared with untreated cells treated with sense oligonucleotides. The reduction in cell number was significant as determined by the Student's t-test (P = 0.04). Untreated cells or cells treated with 20 \( \mu \)M 21-mer sense oligonucleotide underwent 4.5 doublings, while cells treated with antisense oligonucleotide underwent two doublings. We also explored whether this antiproliferative effect was dependent on the dose of the antisense oligonucleotides, and the results are shown in Figure 1a. A dose–response relationship of cell growth inhibition was observed when increasing concentrations of 21-mer antisense oligonucleotides were added to the cultured (Figure 1a). A similar result was observed with 15-mer antisense oligonucleotides (Figure 1a). In contrast, the growth of cells treated with sense strand oligonucleotides at concentrations of 20 \( \mu \)M or less was equivalent to that of untreated cells. At a concentration of 40 \( \mu \)M, however, a 29% inhibition of growth was observed with the 15-mer sense strand, and a 15% inhibition of growth was observed with the 21-mer sense strand.

To be sure that the cell growth inhibition by erbB-2 antisense oligonucleotides was not an idiosyncratic response of SK-BR-3 cells, we used other cell lines with erbB-2 amplification to test the antiproliferative effect of erbB-2 antisense oligonucleotides. Similarly to the results observed in SK-BR-3 cells, 60% growth inhibition was observed in the BT-474 cell line at an antisense oligonucleotide concentration of 20 \( \mu \)M (Figure 1b). BT-474 cells treated for 7 days with 20 \( \mu \)M erbB-2 antisense oligonucleotides increased cell number by a factor of 1.1, while cells treated with the same concentration of sense oligonucleotide underwent a 2.5-fold increase. erbB-2 antisense oligonucleotide concentrations of 100 \( \mu \)M induced a 30% inhibition of growth in the lines MDA-MB-453 and MDA-MB-361, which also have amplification of erbB-2, but there was no apparent inhibitory effect on these cell lines by the sense strand oligonucleotides at 100 \( \mu \)M oligonucleotide concentrations. Since uptake of oligonucleotide was similar in all the cell lines tested, differences in cell line sensitivity to erbB-2 antisense oligonucleotides may be due to variation in the growth dependance of a particular cell on the erbB-2 oncogene signal.

We used MDA-MB-468 and MCF-7 cell lines, which do not have erbB-2 amplification, as controls to study the specificity of the effects of erbB-2 antisense oligonucleotides on erbB-2 overexpressed breast cancer cells. MDA-MB-468 cells have undetectable levels of erbB-2 and overexpress the epidermal growth factor receptor gene. MCF-7 cells have low levels of erbB-2, as assessed by flow cytometry. Both cell lines were shown to have an uptake of FITC-labelled oligonucleotide similar to the cell lines with erbB-2 amplification (Table 1). We observed no differences in cell growth of MDA-MB-468 cells when antisense and sense erbB-2 oligonucleotide treatments were compared (Figure 1c). Dose-related nonspecific toxicity was seen in this cell line to the degree that cell growth was inhibited 20% at 100 \( \mu \)M of either sense and antisense oligonucleotides. Similarly, no sequence-specific effects of erbB-2 antisense oligonucleotides were observed in MCF-7 cells.

To examine whether the antiproliferative effects of erbB-2 antisense oligonucleotides were reflected by changes in cellular DNA synthesis, we assayed short-term incorporation of \([\text{H}]\)thymidine into DNA after exposure to 15-mer oligonucleotides. As shown in Figure 2, thymidine incorporation into DNA was inhibited in SK-BR-3 cells by the erbB-2 antisense oligonucleotides in a dose-dependent manner. The control sense strand had no effect on DNA synthesis at concentrations up to 10 \( \mu \)M, but caused non-specific inhibition of thymidine incorporation at 40 \( \mu \)M, which was consistent with the oligonucleotides toxicity threshold for SK-BR-3 cell growth. Differences in \([\text{H}]\)thymidine incorporation were not due to competition of free thymidine resulting from oligonucleotides degradation, since the number of thymidine residues in both the sense and antisense 15-mer erbB-2

Table 1 Uptake of fluoresceinated oligonucleotide by the different cell lines used in the study

| Cell line   | Mean fluorescence | Median cell size (\( \mu \)m) |
|------------|------------------|-----------------------------|
| SK-Br-3    | 88               | 30                          |
| BT-474     | 60               | 28                          |
| MDA-453    | 77               | 23                          |
| MDA-361    | 72               | 25                          |
| MDA-468    | 65               | 20                          |
| MCF-7      | 72               | 32                          |

*Estimated using 10 \( \mu \) beads (Coulter, Hialeah, FL, USA).
oligonucleotides was the same. As seen in Figure 2, antisense oligonucleotide concentrations as low as 5 μM were effective at inhibiting [3H]thymidine incorporation, while 20 μM was the lowest concentration at inhibiting cell growth. Since the thymidine incorporation experiment was done over a time course of 4 h and cell number in the growth experiment was counted after 7 days, these two results cannot be compared directly. The efficacy of the oligonucleotides in culture may diminish over 7 days even though we could not demonstrate appreciable degradation in serum-free medium.

**Inhibition of erbB-2 protein expression**

The effect of erbB-2 antisense oligonucleotides on cellular erbB-2 protein expression was assessed immunohistochemically and quantitated using an computerised image analysis system. Breast cancer cells with erbB-2 amplification were incubated for 6 days with 21-mer erbB-2 oligonucleotides. erbB-2 staining can be observed in Figure 3. Untreated SK-Br-3 cells exhibited a clear membrane staining for the erbB-2 protein. The erbB-2 staining index of SK-Br-3 cells treated

![Figure 2](image)

**Figure 2** DNA synthesis of SK-Br-3 cells treated with erbB-2 oligonucleotides (○, sense; ●, antisense). SK-Br-3 cells growing exponentially in a 24-well plate were incubated for 4 h in a defined serum-free medium containing increasing concentrations of 21-mer erbB-2 antisense and sense oligonucleotides. The medium was then replaced with 250 μl of 10% FCS-IMEM supplemented with 10 μCi ml⁻¹ of [3H]thymidine. After 3 h, cells were harvested, sonicated and DNA was precipitated with 10% trichloroacetic acid. Error bars represent the standard deviation of three parallel determinations. Data points that appear to lack error bars have standard deviations that are within the range of the data point. DPM, dots per minute.

![Figure 3](image)

**Figure 3** Immunohistochemical appearance of SK-Br-3 cells incubated for 6 days with erbB-2 oligonucleotides. The colour red indicates staining for the erbB-2 oncoprotein, as described in the Material and methods section. Top left: Control cells not treated with oligonucleotides. Top right: Cells treated with sense erbB-2 oligonucleotides. Bottom left: Cells treated with nonsense erbB-2 oligonucleotides. Bottom right: Cells treated with antisense erbB-2 oligonucleotides. The last panel shows a marked reduction of erbB-2 stain.

| Concentration (μM) | erbB-2 staining index* (%) |
|-------------------|---------------------------|
| Control           | 0.9 (100)                 |
| Antisense         | 0.8 (98)                  |
| 5                 | 0.75 (83)                 |
| 20                | 0.6 (66)                  |
| 50                | 0.5 (55)                  |
| Sense             | 1.4 (156)                 |
| 5                 | 1.28 (142)                |
| 20                | 1.5 (167)                 |
| 50                | 1.3 (144)                 |

*Relative to staining of SK-Br-3 cells growing sparsely.
with 20 μM erbB-2 antisense oligonucleotides was approximately 60% that of untreated cells (Table II). The erbB-2 protein levels were inhibited in a dose-dependent manner by erbB-2 antisense oligonucleotides, with a 50% reduction obtained with 50 μM. Comparatively, the sense oligonucleotides did not decrease erbB-2 levels at 50 μM.

We consistently observed enhanced erbB-2 staining index in cells treated with sense oligonucleotides. This same effect was seen when a scrambled-sequence nonsense oligonucleotide was used (Figure 3 and data not shown). We were unable to explain this non-specific effect, which did not correlate with oligonucleotide concentration. However, apparent enhancement of the staining reaction by oligonucleotides suggests that the magnitude of the inhibitory effect of antisense oligonucleotides may be an underestimate of the actual effect.

Discussion

Our experiments provide evidence that erbB-2 overexpression is important for the in vitro proliferation of human breast carcinoma cell lines derived from tumours with an amplified erbB-2 gene. The decrease in erbB-2 protein levels induced by erbB-2 antisense oligonucleotides significantly reduced cellular growth and thymidine incorporation into DNA of these breast cancer cell lines. This antiproliferative effect was specific, since control oligonucleotides, which did not reduce erbB-2 protein, had no effect on cellular growth or DNA synthesis. The specificity of the effect of erbB-2 antisense oligonucleotides was further demonstrated by their lack of effect on MDA-MB-468 and MCF-7 cells, which do not express or express low levels of erbB-2.

erbB-2 antisense oligonucleotides did not arrest completely the growth of the cells overexpressing erbB-2. In our experiments we observed a maximum of 60% inhibition of growth. We generally treated our cultures once with oligonucleotides and measured growth at 7 days. Since intracellular degradation of oligonucleotides occurs, a daily treatment may have achieved a greater degree of cell growth inhibition. Furthermore, the cell lines used in these experiments contained other genetic alterations in addition to erbB-2 amplification, which also may drive cell growth independent of erbB-2. For example, SK-Br-3 cells express c-fms and CSF-1 and have an amplification of the c-myc gene (Horiguchi et al., 1988).

MDA-MB-361 and MDA-MB-363 cells have erbB-2 (Kraus et al., 1989; Plowman et al., 1990). BT-474 and MDA-MB-361 cells express oestrogen and progesterone receptors and manifest a growth response to oestriadiol treatment in vitro and also when grown in nude mice (R. Colomer & E.P. Gelmann, unpublished data). A third possible explanation for our results is the existence of cell line heterogeneity. Preliminary evidence from 20 clonal derivatives of SK-Br-3 cells shows that the antiproliferative effects of erbB-2 antisense oligonucleotides are retained by less than 50% of the clones, and that this is not related to loss of expression of erbB-2 protein. A recent report has suggested that a subline of the lymphoma cells DHL-4 develops resistance to an antisense oligonucleotide by the emergence of intracellular degradation that is absent in the parental line (Ryte et al., 1993). Therefore there may be cellular heterogeneity regarding sensitivity to oligonucleotides, and resistance to the agents may be a possible future experiment. Nevertheless, our finding that erbB-2 antisense oligonucleotides inhibit the growth of cells overexpressing erbB-2 by 50–60% is consistent with a similar inhibition of proliferation observed in SK-Br-3 cells with erbB-2 antibodies (Hudziak et al., 1989).

We have shown that erbB-2 antisense oligonucleotides induce a 35% decrease in erbB-2 protein staining. Cells treated with sense erbB-2 oligonucleotides, in contrast, did not decrease erbB-2 levels and rather showed an increase in erbB-2 protein staining. The specificity of the effect of antisense oligonucleotides was further substantiated by the fact that we observed no reduction in erbB-2 staining by erbB-2 sense oligonucleotides, even at concentrations at which these compounds had non-specific antiproliferative effects. The erbB-2 staining increase induced by erbB-2 sense oligonucleotides does not seem to be specific for the sense sequence that we used, since it did not correlate with the concentration of oligonucleotide and, furthermore, other unrelated oligonucleotides induced a similar small increase in erbB-2 staining (data not shown). erbB-2 antisense oligonucleotides decreased erbB-2 levels at the cell membrane without a concurrent increase in intracellular stain, which is consistent with a decreased erbB-2 protein synthesis. This contrasts with the down-regulating effect of the erbB-2 ligand gp30 on cells overexpressing erbB-2. gp30 decreases membrane-associated erbB-2, but partly at the expense of increased intracellular accumulation of erbB-2 protein (Bacus et al., 1992).

By inhibiting the expression of erbB-2 protein with antisense oligonucleotides we have shown that breast cancer cells with erbB-2 amplification are subject to specific growth inhibition. Our results support the hypothesis that erbB-2 oncogene amplification confers a growth advantage on cells (Di Fiore et al., 1987; Hudziak et al., 1987). Other procedures that decrease erbB-2 levels interfere with cellular proliferation. The selective pressure provided by very high erbB-2 receptor levels can be reversed using monoclonal antibodies to erbB-2 (Drebin et al., 1988; Hudziak et al., 1990). In addition, we have reported that elevated concentrations of gp30 are growth inhibitory in cells with elevated expression of erbB-2 (Lupu et al., 1990). Clinically, erbB-2 is overexpressed in 25% of human primary breast carcinomas, and it is correlated with an adverse prognosis. The experimental data strongly suggest that the erbB-2 oncogene and its product can be potential therapeutic targets in a significant fraction of breast carcinomas. Therapeutic strategies aimed at the erbB-2 gene may have a relatively high chance of success. We make this speculation based on the fact that two cancer cell lines without erbB-2 overexpression showed no growth effects after erbB-2 antisense oligonucleotides treatment and, similarly erbB-2 antibodies and gp30 had no antiproliferative effects on cells without overexpression (Hudziak et al., 1989; Lupu et al., 1990). This theoretical approach is further supported by the observations that there is homogeneous overexpression of erbB-2 in breast carcinomas, that erbB-2 is overexpressed in tumours that overexpress the oncogene (Iglehart et al., 1990), while the surrounding mammary tissue is essentially void of erbB-2 overexpression, as are other normal tissues throughout the body (Press et al., 1990). Antisense oligonucleotides chemical analogues synthesised to confer relative serum and cellular nuclease resistance represent a new class of compounds with potential application for gene-specific therapeutic intervention (Tidd, 1990). Experiments with these agents in vivo will help define the spectrum of applications for oligonucleotides and their derivatives.

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