ErbB-4 May Control Behavior of Prostate Cancer Cells and Serve as a Target for Molecular Therapy

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PURPOSE. To assess ErbB-4 expression in advanced human prostate cancer (PC) cell lines, the role of ErbB-4 in motility, migration, and proliferative/tumorigenic potential of PC cells, and efficacy of anti-ErbB-4 monoclonal antibody (Mab) treatment on PC cells in vitro and tumor growth in vivo.

MATERIALS AND METHODS. Established advanced human PC cell lines (PC-3, CI-1, and Du-145) were evaluated for ErbB-4 expression. Several CI-1 cell line clones expressing various levels of ErbB-4 were isolated, their motility, migration capacity, and in vitro proliferation as well as survival following Mab treatment were evaluated. Tumorigenicity and proliferation capacity of these clones in vivo and efficacy of Mab treatment on tumor growth were estimated by measurements of subcutaneous tumors developed in nude mice.

RESULTS. PC cell lines studied express ErbB-4. Both PC-3 and Du-145 cell lines express high ErbB-4 levels; only 50% of CI-1 cells express ErbB-4 with large heterogeneity. CI-1 sub-clones highly expressing ErbB-4 showed increased cell motility, migration, and proliferation rate in vitro and enhanced growth in vivo, compared to clones with low ErbB-4 expression. Mab treatment inhibited the growth of cells expressing high but not low ErbB-4 levels in vitro and decreased the growth of subcutaneous tumors in nude mice generated by ErbB-4 highly expressing cells.

CONCLUSIONS. High expression of ErbB-4 in prostate cancer CI-1 cell clones correlated with high proliferative and migration capacity and high tumorigenic potential. The inhibitory effect of Mab on cell proliferation and on subcutaneous tumor growth suggests ErbB-4’s potential as a target for molecular anticancer therapy. Prostate 67: 871–880, 2007. © 2007 Wiley-Liss, Inc.

KEY WORDS: prostate cancer; ErbB-4; tumorigenicity; targeted molecular therapy
advanced PC, but long-term androgen ablation eventually results in androgen-independent (AI) tumor cell growth [1]. A limited number of chemotherapeutic regimens have proved to be effective in reducing prostate-specific antigen (PSA) levels and symptoms of AI PC [2,3]. Although the recent introduction of Taxotere to the therapeutic armamentarium has increased survival by 2–3 months [4,5], the median survival of AI PC is less than 2 years [6]. The mechanism of AI tumor progression remains obscure, but it might be associated with alternative growth signaling [1,7,8]. As such, targeting signal transduction proteins [9–13] may provide a new approach for treating PC.

One of the most important pathways involved in cancer regulation is the signaling pathways regulated by the ErbB receptor family. Growth factor receptors of this family were found to be over-expressed in different tumor tissues [14], including those of PC [15,16]. The structure of the ErbB-4 receptor and its mechanism of action are similar to those of ErbB-1, but much less is known about its role in carcinogenesis [17]. Some studies correlated the over-expression of ErbB-4 with enhanced cell proliferation [14,15], inhibition of apoptosis [16], poor tumor control and decreased response to chemotherapy [18–21]. Others claimed that ErbB-4 has a tumor suppressive effect [22,23].

The role of ErbB-4 in the biology of PC cells and their tumorigenicity, as well as in the outcome of PC patients remains undefined. ErbB-4 expression in PC specimens and cultured cells is a controversial issue. Several studies [15,16,24,25] and our own clinical data [26] demonstrated the presence of over-expression of ErbB-4 in one-third of PC patients, while Robinson et al. [27] did not find evidence of ErbB-4 in any of the prostate tumors that they tested. Several authors [27,28] did not observe ErbB-4 over-expression in cultured PC cells (Du-145, PC-3, LNCaP), while Siegall et al. [29] demonstrated high levels of ErbB-4 in LNCaP cells.

The aims of this study were (i) to assess ErbB-4 expression in advanced human PC cell lines, (ii) to evaluate a role of ErbB-4 in the motility and migration capacity of PC cells, as well as in their proliferative and tumorigenic potential, and (iii) to test the efficacy of anti-ErbB-4 antibody treatment on PC cells in vitro and in vivo.

**MATERIALS AND METHODS**

**Cell Lines and Culture Conditions**

Three human PC cell lines, PC-3, Cl-1, and Du-145, were kindly provided by the Israel Prostate Cancer Foundation cell facility (Z. Eschar, Weizmann Institute of Science, Rehovot, Israel). All the cell lines were maintained in DMEM supplemented with 10% heat-inactivated FCS, antibiotics, glutamine, 0.1 mM non-essential amino acids and 1.0 mM sodium pyruvate (Biological Industries, Beit Haemek, Israel) and were grown in 5% CO2 at 37°C in a water-jacketed incubator with 100% humidity. The cells were harvested by trypsin solution with 1–2 passages per week in a split ratio of 1:3–5, and 24-hr cell cultures were used in all the experiments.

**Antibodies**

Polyclonal anti-ErbB-1-4 antibodies were purchased from Santa Cruz (CA), and monoclonal antibody (Mab) to the extracellular domain of ErbB-4 was obtained from the mouse hybridoma H.72.8 (developed in the laboratory of Y. Yarden, Weizmann Institute of Science, Rehovot, Israel) [30]. This antibody was prepared in mouse ascites that was purified using Protein-G-Sepharose column 4B Fast Flow (Sigma, Israel) and was used for both the in vitro and the in vivo experiments.

**Limited Dilution Cell Cloning**

Tumor cells were seeded on 96-well plates, each containing one cell per well. The cells were expanded in these wells for a 2-week period and then transferred individually to culture flasks. The resultant clones were stored and screened for ErbB-4 expression by immunohistochemistry (IHC) and Western blot assay.

**Immunohistochemistry (IHC)**

Cell pellets and tissue samples were formalin-fixed and embedded in paraffin for routine (hematoxylin and eosin—H&E) and immunohistochemical (ErbB-4) staining. Antigen retrieval was performed at 95°C in citrate buffer pH 6.0, 6.4 M sodium citrate dehydrate, 1.6 M citric acid monohydrate for 40 min. The slides were cooled at room temperature for 20 min and washed 3 × 3 min with Tris-buffer pH 7.6, 0.15 M sodium chloride, 0.05 M Trizma HU. The slides were peroxidase blocked for 5 min and washed as above. They were then incubated for 30 min with the primary antibody, followed by the secondary antigen (visualization reagent), followed by the substrate-chromogen solution (3,3’-diaminobenzidine), and finally counter-stained with hematoxylin. Paraffin sections of normal skeletal muscles known to express ErbB-4 were used as positive control, and the primary antibodies were removed for negative control. Staining was quantified between “0” and “3+” according to the Dako instructions.

**Immunoprecipitation and Western Blot Analyses**

**Preparation of cell lysates.** The cells were grown in 10 cm tissue culture dishes, washed briefly with ice-cold
Immunoprecipitation. Fifteen microliters of beads (Protein G-Sepharose, Sigma) for each sample (i.e., a lysate originating from one variant of the experiments) were washed three times with HNTG buffer containing 20 mM HEPES pH 7.5, 150 mM NaCl, 0.1% Triton X-100, 10% glycerol. The antibody was coupled to the beads for 1 hr at 4°C while shaking. Lysates were added to beads/antibody conjugates and incubated overnight at 4°C while shaking. Immunoprecipitates were washed three times with HNTG buffer, mixed with sample buffer, heated for 5 min at 95°C and subjected to SDS–PAGE electrophoresis (7.5%).

Western blot. After electrophoresis, proteins were electrophoretically transferred to nitrocellulose membranes (1–2 hr, 4°C) and then saturated overnight at room temperature in a blocking solution (TBST: 50 mM Tris-HCl, pH 7.5, 150 mM NaCl, and 0.1% Tween 20 with the addition of 5% low fat milk). The membranes were then incubated for 1.5 hr at room temperature with the first antibody (rabbit polyclonal IgG) diluted in TBST containing 2% low fat milk. The membranes were washed thoroughly with TBST solution and incubated for 1 hr at room temperature with conjugated goat anti-rabbit IgG-HRP. Signals were detected by using the enhanced chemo-luminescence method (EZ-ECL, Biological Industries).

Cell Motility Assay

Cell motility was evaluated by a capillary method as described by Harrington and Stastny [31]. The cells were pre-cultivated for 24 hr in full growth medium. Capillary hematocrit tubes (75 mm long with a 1.2 mm inner diameter) were filled with the suspension of tumor cells and then sealed and centrifuged at 1,000 rpm for 3 min. After centrifugation, the tubes were cut at the cell-fluid interface, imbedded in a drop of silicone grease and positioned in Sykes–Moore chambers filled with the full growth medium. Migration areas were determined after 24 and 48 hr of incubation at 37°C by tracing a projected image of the cells on transparent film and cutting and weighing them. Each variant of the experiment was done in triplicate and repeated 2–3 times.

Migration Assay

Cell migration was assessed by a 48-well Boyden microchemotaxis chamber technique. The cells used in this analysis were pre-cultivated 24 hr in full growth medium. The lower compartment of the chamber was loaded with 30 µl of the attractive medium (i.e., the conditioned medium after 24 hr of cultivation of clone 6 of Cl-1 cells). The upper compartment of the chamber was loaded with 50 µl of the cells (2 × 10^5 cells/well), re-suspended in 0.5% BSA in DMEM medium. Two compartments were separated by an 8 µm pore-sized polycarbonate filter (Neuro Probe, Inc.). Following 6 hr of incubation at 37°C, the filter was removed. The cells migrating through to the underside of the filter were fixed and stained with Giemsa solution and then counted in five fields by light microscopy. Each variant of the experiment was done in triplicate and repeated 2–3 times.

Colorimetric Tetrazolium Salt (XTT) Assay for the Number of Viable Cells

Cell density was evaluated by colorimetric assay based on the conversion of XTT to orange-colored formazan compounds by cellular dehydrogenases. The advantage of this assay with the use of 96 micro-well plates is in the ability to rapidly test numerous arms of each experiment in the same conditions. Typically, 200 µl with a known number of cells from exponentially growing cultures were plated in 96 micro-well flat-bottom plates. For evaluation of the growth rate, the cell density was measured every 24 hr during several days of incubation. To determine the effect of Mab on cell survival, the treatment was started following 24 hr of cell culturing. Mab was added in varying concentrations to each of three replicate wells and incubated for 4 days. The effect of Mab on cell survival was calculated by comparing the density of intact cells to the cells exposed to them. Cell density was determined by the XTT assay as follows. A freshly prepared mixture of XTT and an activation reagent (PMS) was added into each well (50 µl). Following 2 hr of incubation at 37°C, the plates were placed on a mechanical plate shaker of a computerized automatic microwell plate spectro-photometer and shaken for 30 sec, and the optical densities (ODs) of the dye were read at 450 nm. The measurements were repeated following 4 and 6 hr of incubation. The time point of the assay with optimal OD readings was chosen to assay the cell number. When more than one time point fitted these criteria, the results in the different time points were normalized and averaged. The OD readings were shown to correlate well (r > 0.97–0.99) with the number of seeded cells/well.
Animals

The 8- to 10-week-old female athymic CD-1 nude mice were obtained from the Harlan Animal Production Area (Jerusalem, Israel). The mice were housed in a laminar airflow cabinet under specific pathogen-free conditions in standard vinyl cages with air filter tops. Cages, bedding, and water were autoclaved before use. All facilities were approved by the Institutional Animal Care and Use Committee in accordance with the current regulations and standards of the Israeli Ministry of Agriculture and the Israeli Ministry of Health.

Assay of Tumor Growth in Nude Mice

The ErbB-4-positive and ErbB-4-negative clones of Cl-1 tumor cells (2–3 × 10^6 cells/0.2 ml saline) were injected subcutaneously (s.c.) into flank areas of CD-1 athymic nude mice. Ten mice were used for each variant of the experiment that was repeated at least twice. The tumor size (the average taken from two perpendicular diameters) was measured twice weekly using digital caliper. The mice were autopsied when they became moribund or at 4–6 weeks after s.c. cell injection. The internal organs were removed, rinsed in water, fixed in 10% formalin and examined under a dissecting microscope. Developed tumors underwent pathologic evaluation.

To test the efficacy of Mab treatment on the growth of tumors developed by ErbB-4-positive Cl-1 cells, the antibody (0.6 mg/0.2 ml per mouse) was injected intraperitoneally twice weekly, starting 1 week after cell implantation.

Assay for Experimental PC Metastasis

Tumor cells suspended in 0.2 ml of saline were injected through the lateral tail vein of the nude mice. Eight weeks later, the mice were euthanized, autopsied, and studied under a dissecting microscope. All organs suspected of having metastases were examined histopathologically.

Statistics

The results for each variant in the different experimental designs were represented as an average of 2–4 experiments; each arm was typically performed in triplicate. Mean values and standard errors were calculated for each point from the pooled normalized data.

Comparison between the various clones by the examination of time trend and evaluation of treatment efficacy on tumor growth were performed by way of One-way Analysis of Variance with repeated measures using the mixed model. Whenever a main effect was found significant, pair wise comparisons between the levels of those effects were done using Hochberg’s GT2 method for multiple comparisons. The significance of difference in the treatment effect on the cell clones and the cell lines in vitro was evaluated by t-test.

RESULTS

Expression of ErbB-4 in Prostate Cancer Cells

ErbB-4 expression in cultured prostate cancer cells was evaluated using IHC and Western blot analysis (Fig. 1). Both methods revealed significant expression...
of ErbB-4 (mostly in the cytoplasm) in all three PC cell lines tested. Maximal ErbB-4 expression was found in Du-145 cells. An immunoprecipitation assay confirmed these results (data not shown). Immunohistochemistry detected extensive heterogeneity for ErbB-4 staining in Cl-1 cells. This cell line was used for isolation of cell clones displaying different expression levels of ErbB-4.

**ErbB-4 Expression in Selected Clones of the Cl-1 Cell Line**

To assess whether the level of ErbB-4 expression influences cell behavior, several clones from Cl-1 cell line were isolated by using a limited dilution technique. The expression of all four ErbB receptors was evaluated using Western blot analysis (Fig. 2). The cell clones obtained from Cl-1 cells demonstrated different levels of ErbB-4 expression: a low level of ErbB-4 was found in clones 2 and 7 while clones 3 and 6 were ErbB-4 over-expressed. The expression of ErbB-1 was significantly higher in clones 2 and 3. ErbB-2 and ErbB-3 were similarly expressed in all tested clones.

**Motility and Migration of Cl-1 Clones With Different ErbB-4 Expression**

The motility and migration capacity of the Cl-1 clones with different levels of ErbB-4 expression were assessed in order to characterize a possible role of ErbB-4 expression in metastatic ability. The rate of cell motility was measured by the amount of cells that moved out of a capillary bed in a period of 24 or 48 hr. As is shown in Figure 3B, a higher rate of motility was seen in ErbB-4-expressing clone 3 compared to low ErbB-4-expressing clone 2 within a period of 24 hr as well as after 48 hr. But the difference between the clones in the motility rate was not statistically significant.

Cell migration was tested by employing the Boyden microchemotaxis chamber technique using an 8 μm pore-sized polycarbonate filter and conditioned medium (the medium collected after 24 hr of cultivation of clone 6 of Cl-1 cells) as the attractant. All the clones demonstrated migration ability, but the clones with high ErbB-4 expression (clones 3 and 6) migrated more efficiently than the clones 2 and 7 with low level of ErbB-4 (Fig. 3C).

**Effect of an Anti-ErbB-4 Antibody on PC Cell Proliferation**

To further investigate whether ErbB-4 might mediate a growth function, the effect of monoclonal anti-ErbB-4 antibody (Mab) on the proliferation of prostate cancer cells was studied in vitro using Du-145 and PC-3 cells as well as Cl-1 cells clone 3 cells with high level of ErbB-4 and clone 7 with low ErbB-4 expression. Mab treatment inhibited the proliferation of the cells expressing high level of ErbB-4 in a dose-dependent manner (Fig. 4) and it reached a statistical significant difference at a dose of 30 mcg/ml ($P < 0.005$). In contrast, the proliferation of a Cl-1 cells clone 7 expressing low level of ErbB-4 was not affected by Mab at any of the concentrations tested (Fig. 4).

**Behavior of the Cl-1 Clones With Different ErbB-4 Expressions In vivo**

To determine the role of ErbB-4 in the tumorigenic and metastatic capacity of the PC cells, the Cl-1 clones with high and low ErbB-4 expression were implanted s.c. into CD-1 nude mice. No significant differences were discernible in the tumor take between either of the clone types. Examination of tumor growth rates,
Fig. 3. Characteristics of the Cl-1 cell clones. A: Cell proliferation in vitro. The cells (1,000 cells/well) were seeded in 96 micro-well plates in triplicates at the same cell density (1,000 cells/well). Cell density was measured using XTT assay. All cell clones demonstrated an exponential growth. A higher growth rate was seen in the over-expressed ErbB-4 clones (c3 and c6) compared to the non-over expressed (c2 and c7) clones. B: Cell motility in vitro. ErbB-4 over-expressed clone 3 demonstrated a higher rate of motility (evaluated by weighting the image of the area occupied by the cells moved out of the capillary bed) in comparison to the motility of a low ErbB-4-expressed clone 2 at 24 and 48 hr. C: Cell migration in vitro. Boyden chamber technique was used to evaluate the migration capacity of all four clones selected. 2 × 10^5 cells re-suspended in 0.5% BSA in DMEM medium were loaded in the upper compartment of the chamber. Following 6 hr of incubation at 37 °C, the cells that migrated through an 8 μm pore-sized filter were fixed, stained with Giemsa solution and counted. The over-expressed clones (c3 and c6) migrated more efficiently than the non-over-expressed (c2 and c7) clones. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

Fig. 4. Effect of anti-ErbB-4 monoclonal antibody (Mab) on proliferation of Du-145, PC-3, and Cl-1 cells. The cells were seeded in 96 micro-well plates in triplicates at the same cell density (1,500 cells/well), exposed to different concentrations of Mab for 72 hr and then their density was measured by XTT assay. Cell survival was calculated by comparing the density of the intact (100% survival) and the treated cells. A dose dependent inhibitory effect of Mab was found in the over-expressed cells (Du-145, PC-3, and c3 derived from Cl-1 cells) while a less inhibitory effect was seen in the c7 of Cl-1 cells that has a less over-expressed ErbB-4. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The Prostate DOI 10.1002/pros
however, demonstrated clearly different results. By day 51 after injection, the average size of the tumors produced by the clone with high ErbB-4 expression was >17 mm in diameter, whereas tumors produced by the clone with low ErbB-4 expression did not exceed 11 mm in diameter (Fig. 5A). The growth rate of the tumors was directly correlated with the clones’ propensity to express ErbB-4. The clone with low ErbB-4 expression produced slow-growing tumors, whereas the clone with high ErbB-4 expression produced rapidly growing tumors (Fig. 5A) \( (P = 0.0225) \). Macroscopic evaluation of s.c. formations found irregular, infiltrating masses. Microscopy of these primary tumors revealed of poorly differentiated high-grade, anaplastic tumor cells surrounding by pseudocapsule (Fig. 5B). Acinar formation was absent, and areas of necrosis were evident. At microscopic examination mitotic figures were conspicuous and there was considerable variation in cellular and nuclear pleomorphism. Severe nuclear anaplasia was present with evidence of hyperchromatic, large nuclei associated with a course chromatin pattern. Large, abnormal nucleoli were frequently encountered. Local infiltration and invasion into surrounding muscles were observed. IHC of the tumors developed from the high ErbB-4 clone revealed that the cancer cells retained high level of ErbB-4 (Fig. 5C). Interestingly, no spontaneous metastases were found despite the growth of Cl-1 cell implants into large tumors. Nor intravenous injection of the tumor cells of either clone resulted in metastases (data not shown).

**Effect of Mab on Growth of Tumors Developed by Cl-1 Cells With High ErbB-4**

ErbB-4-positive Cl-1 cells were implanted s.c. in CD-1 nude mice in order to test the efficacy of an anti-ErbB-4 antibody treatment on tumorigenic growth of PC cells in vivo. After 1 week, when a tumor size reached an average 3–5 mm in diameter, the treatment group received Mab (0.6 mg/mouse twice weekly) while the control group was injected with saline. Five injections were delivered in 2.5 weeks. As shown in Figure 6, the Mab treatment significantly inhibited the growth of PC tumors developed by CI-1 cells with high ErbB-4 expression \( (P < 0.0001) \).

![Figure 5](https://example.com/figure5.png)

*Fig. 5.* Development of the subcutaneous tumors in CD-1 nude mice from the Cl-1 clone 3 (that over-expressed ErbB-4) and clone 7 (had non over-expressed ErbB-4). **A:** Growth of the tumors generated by c3 and c7 cells. The cells \( (3 \times 10^6/0.2 \text{ ml PBS}) \) were injected to the flank of nude mice. Tumor size was measured twice a week. Each point represents an average data of 8–10 tumors. Tumors generated by c3 (highly expressed ErbB-4 cells) grew up faster than tumors generated by c7 cells. **B:** Microscopy of primary tumors generated by c3 and c7 (H&E staining). Poorly differentiated, high-grade anaplastic hyperchromatic tumor cells surrounded by pseudocapsule were found; cellular and nuclear pleomorphism as well as large, abnormal nucleoli were frequently encountered. **C:** ErbB-4 expression in primary tumors generated by c3 and c7 cells. The c3 cells but not C7 retained the over-expression of ErbB-4 (seen as brown staining and located mostly in cytoplasm). [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
Microscopic examination of tumor sections from mice that received Mab treatment revealed multiple apoptotic cells with piknotic and fragmented nuclei, karyorexis, and loss of cytoplasm (data not shown).

**DISCUSSION**

The expression of ErbB-4 and its role in PC cell biology continue to be controversial issues [15,16] [24–29]. Some reports claim that there is no ErbB-4 expression in PC cells [27,28]. Our findings in the current study refute this notion: we used three different experimental methods (IHC, Western blot, and immunoprecipitation assays) and could demonstrate (Fig. 1) high levels of ErbB-4 expression in three widely used PC cell lines (Du-145, PC-3, and Cl-1). In addition, we found that the pattern of expression seen for ErbB-4 by IHC was generally concordant with the protein expression measured by Western blot. Our findings are in good agreement with the reported data on high levels of ErbB-4 in LNCaP cells [29].

We demonstrated a direct relationship between a high expression of ErbB-4 and the proliferation rate of Cl-1 cells. We found that the Cl-1 clones with high Erb B-4 expression had an increased proliferation rate, whereas the clones with low ErbB-4 expression showed a decreased proliferation (Fig. 3A). Evidence that Erb B-4 can stimulate growth has been reported in several studies [32–34]. In a number of ectopically ErbB-4-expressing cell lines, neuregulin-activated ErbB-4 provoked DNA synthesis [32] and cell division [33]. Another study [34] employed ErbB-4 rybozymes, which target specific sites within the ErbB-4 mRNA to cleave it and thereby inhibit cell proliferation.

Contrarily, other studies supposed that ErbB-4 can prevent tumor growth by inducing cell differentiation (expression of milk protein and fat droplets in breast cancer cells) [35] and neurite outgrowth in PC12 neuronal cells [36]. The existence of at least four functionally different isoforms of ErbB-4 due to alternative mRNA splicing [33] might explain part of this controversy. These isoforms contain alternative extra-cellular sequences which determine either sensitivity (JM-a) or insensitivity (JM-b) to a proteolytic cleavage [36] and therefore have a different biological role in cancer biology.

Using a capillary assay and the Boyden chamber, we found that increased cell motility (Fig. 3B) and migration (Fig. 3C) correlates with high expression of ErbB-4 in Cl-1 clones. We believe this to be the first demonstration of an association between the motility of PC cells and the expression of ErbB-4. Our results are consistent with other in vitro and in vivo studies in showing that ErbB-4 expression can enhance the motility of normal and tumor cells [36–38]. It was recently reported that ErbB-4 is expressed in neurons migrating from the ganglionic eminences [37] and cranial neural crest [38], as well as in other neural progenitor cells [39]. Several studies suggested that the ErbB-4 ligand neuregulin (NRG) may be involved in actin reorganization, formation of lamellipodia, membrane ruffling, and cell migration [40–43]. No metastases, however, were found in a spontaneous or experimental metastasis assay when we used either high or low ErbB-4 expressing Cl-1 clones. Among the possible explanations for this phenomenon is that of a multiplicity of steps being involved in the metastatic process, including growth of a primary tumor, invasion, survival in blood stream, extravasation, and arrest and growth in secondary sites. Failure to complete even one of these steps may prevent metastasis [44].

We sought a role of ErbB-4 expression in the tumorigenicity of PC cells in a mouse model by implanting Cl-1 clones that had different levels of ErbB-4 expression. Both clones demonstrated tumorigenicity capacity, but tumors developed much faster from the cells with a high expression of ErbB-4 than from the cells with a low ErbB-4 expression (Fig. 5A). These data emphasize the oncogenic properties of ErbB-4 in the studied PC cells.

We also investigated whether blocking of ErbB-4 would inhibit proliferation of PC cells in vitro and in vivo. The in vitro incubation of the Cl-1 cells with a Mab to ErbB-4 decreased the survival of clones with high Erb-4 expression in a dose-dependent manner, but it did not affect the survival of clones with low ErbB4 expression (Fig. 4). Similar results were found in in-vivo experiments that showed that Mab treatment...
inhibited the growth of tumors with high ErbB-4 expression compared to a control arm (Fig. 6). These findings lead us to propose that ErbB-4 may serve as a target for molecular therapy of prostate cancer.

In conclusion, ErbB-4 is highly expressed in the studied human PC cell lines. The high level of ErbB-4 expression in CI-1 cell clones correlated with high proliferative and migratory capacity, as well as with high tumorigenic potential. The inhibitory effect of an anti-ErbB-4 Mab on ErbB-4-positive cells in vitro and in vivo suggests that ErbB-4 may serve as a target for molecular therapy in PC patients.

ACKNOWLEDGMENTS

Esther Eshkol is thanked for editorial assistance.

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