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Research Paper

Expression of Basic Fibroblast Growth Factor Correlates with Resistance to Paclitaxel in Human Patient Tumors

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Background. Preclinical results indicate acidic fibroblast growth factor (aFGF) and basic FGF (bFGF) present in solid tumors as a cause of broad-spectrum chemoresistance, whereas earlier clinical studies suggest that bFGF expression is associated with opposing outcomes in patients. We investigated the relationship between FGF expression and paclitaxel activity in tumors from bladder, breast, head and neck, ovarian, and prostate cancer patients.
Materials and Methods. Tumors (n = 96) were maintained in three-dimensional histocultures, retaining tumor–stromal interaction. Bladder tumors were treated with paclitaxel for 2 h, and the other tumors for 24 h. Antiproliferative and proapoptotic effects of paclitaxel were quantified and correlated with expression of aFGF, bFGF, P-glycoprotein (Pgp), p53, and bcl-2.
Results. Fifty-one percent (49/96) and 63% (61/96) of tumors showed aFGF and bFGF staining, respectively. aFGF expression was positively correlated with tumor stage ($p < 0.01$), and bFGF expression with tumor grade and Pgp expression ($p < 0.05$). Paclitaxel inhibited antiproliferation in 86% of tumors (83/96), with an average inhibition of 46 ± 19% (mean ± SD) in the responding tumors. Paclitaxel also induced apoptosis in 96% of tumors (92/96), with an average apoptotic index of 12 ± 7% in the responding tumors. aFGF expression did not correlate with tumor sensitivity to paclitaxel, whereas bFGF expression showed an inverse correlation ($p < 0.01$). bFGF expression was a stronger predictor of paclitaxel resistance compared to Pgp, p53, or Bcl-2.
Conclusion. These results support a role of bFGF in paclitaxel resistance in human patient tumors.

KEY WORDS: fibroblast growth factor; paclitaxel; resistance.

INTRODUCTION

Fibroblast growth factors (FGF) constitute a large family of growth factors that are important in the control of cell growth, differentiation, and embryogenesis (1–3). Acidic and basic fibroblast growth factors (aFGF and bFGF) are the most abundant forms and the most extensively studied. bFGF is also a potent angiogenic factor that acts as both a mitogen and an activation of migration for endothelial cells (4).

The role of FGFs in chemoresistance is unclear. Some reports support the view that bFGF protects tumor and stromal cells from chemotherapy or radiotherapy (3,5–13). For example, bFGF prevented etoposide-induced apoptosis in small cell lung cancer H-510 cells, and induced resistance of human bladder tumor cells to cisplatin, chronic lymphocytic leukemia cells to fludarabine, and Hela and endothelial cells to ionizing radiation. bFGF also caused resistance of fibroblasts to *N*-(phosphonacetyl)-L-aspartic acid, muscle cells to sublethal ischemic insult, neural cells to neomycin analog G418, and inhibited apoptosis in vascular smooth muscle cells. In contrast, several reports indicated that addition of exogeneous bFGF or overexpression of bFGF enhanced the sensitivity of human breast MCF7 tumor cells to multiple chemotherapeutic agents (i.e., cisplatin, etoposide, 5-fluorouracil, doxorubicin, carboplatin, and docetaxel) (14–17). The mechanism of chemosensitization is reported to arise from down-regulation of *bcl-2* and up-regulation of *bax* (15,16,18,19). bFGF-mediated chemosensitization was also observed in other cell lines, including human neuronal PC12, NIH313 fibroblasts, and two ovarian and one pancreatic tumor cell lines (14,19,20).

Opposing effects of bFGF expression on patient prognosis have also been reported. On one hand, bFGF expression correlates with poor prognosis in lung, brain, thyroid, liver, and gastric cancer patients (21–26). On the other hand, higher bFGF expression was associated with improved overall and disease-free survival in breast and ovarian cancer patients (27–33). Furthermore, one study showed that lower bFGF plasma levels (<400 pg/mL) in primary breast tumors significantly correlated with increased tumor size and higher tumor stage (32), whereas another study showed no significant relationship between tumor bFGF levels and survival (31), and two more studies showed that enhanced bFGF expression was associated with aggressive disease and worse prognosis in primary, nodal-negative disease (28,33).

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Our laboratory has shown that aFGF and bFGF, at clinically relevant concentrations, induce an up to 10-fold resistance to drugs with diverse structures and action mechanisms (34). Subsequent *in vitro* and *in vivo* preclinical studies showed enhancement of chemosensitivity by inhibitors of these growth factors (34–36). These findings support using the FGF-targeting approach for chemosensitization. The present study was conducted in part to examine the potential clinical utility of such an approach and in part to address the ambiguity introduced by the earlier, contradicting results on the role of bFGF on chemosensitivity. For this purpose, we used the histocultures system, which retains the microenvironment of solid tumors that is increasingly recognized as being important in determining chemosensitivity (37,38). The major advantages of the histoculture system are the maintenance of three-dimensional tissue architecture, cell–cell interaction, intratumoral heterogeneity, and the intertumoral heterogeneity when the histocultures are established with materials from different tumor types or hosts (e.g., specimens from different patients). The clinical relevance of the histoculture system had been demonstrated in retrospective and semiprospective preclinical and clinical studies, showing that the chemosensitivity in patient tumor histocultures correlates with the sensitivity, resistance, and survival of head and neck, colorectal, and gastric cancer patients who were treated with mitomycin, doxorubicin, 5-fluorouracil, or cisplatin (39–41).

Using tumors obtained from bladder, breast, head and neck, ovarian, prostate cancer patients, we established the pharmacodynamics of paclitaxel and reported the relationships between paclitaxel activity and tumor pathobiological parameters such as tumor grade, stage and proliferation, p53 status, and expression of the drug efflux *mdr1* P-glycoprotein and the prosurvival Bcl-2 protein (42). The goal of the present study was to extend our previous observations and use the archived tissues to determine whether the expression of aFGF and/or bFGF is an important determinant of paclitaxel resistance in clinical specimens.

MATERIALS AND METHODS

Chemicals and Supplies

The chemicals used to study the pharmacodynamics of paclitaxel and to detect Pgp, p53, and Bcl-2 were as previously described (42). Monoclonal antibodies against aFGF and bFGF were purchased from Sigma (St. Louis, MO, USA), and Labeled Streptavidin–Biotin detection kit from Dako (Carpiteria, CA, USA). All chemicals and reagents were used as received.

Procurement of Tumor Specimens

Specimens of human bladder, breast, head and neck, ovarian, and prostate tumors were obtained via the Tumor Procurement Service at The Ohio State University Comprehensive Cancer Center. Tumor pathology was determined by university pathologists. Of the 96 tumors studied, 95 were from chemotherapy-naive patients. The remaining tumor was from a head and neck cancer patient that had received paclitaxel treatment.

Pharmacologic Effects of Paclitaxel

The pharmacodynamic data of the antiproliferative and proapoptotic effects of paclitaxel were obtained from our previous studies (42). The data were further analyzed to obtain the overall effects (i.e., maximal antiproliferation effect plus maximal apoptotic index) in individual tumors.

The determination of drug effects was as described previously (42). Briefly, patient tumors were cut to about $1\,mm^3$ pieces. This size was found to be optimal for drug activity evaluation (39–41). Four to six tumor pieces were placed on a $1\,cm^2$ collegen gel presoaked in medium in 6-well plates. After 3–4 days, the tumor histocultures were treated with paclitaxel for 2 h (bladder tumors) or 24 h (all other tumors). The 2-h treatment is the duration of intravesical therapy of superficial bladder cancer, whereas the 24-h exposure is one of the commonly used treatment schedules in patients. Proliferating cells were identified by their labeling with a DNA precursor (bromodeoxyuridine or thymidine). Apoptotic cells were identified by morphological changes, TdT-mediated dUTP nick end labeling, and/or DNA fragmentation. The proliferation and apoptotic indices were calculated as the (number of labeled tumor cells) divided by (number of total tumor cells). Inhibition of proliferation was calculated as (difference in proliferation indices between untreated controls and drug-treated samples) divided by (proliferation index of untreated controls).

For the antiproliferation effect, we measured the maximal effect (E_{max}) and the paclitaxel concentration that produced 30% inhibition of DNA precursor incorporation (IC_{30}) in all tumor cells (i.e., viable plus proapoptotic cells). For the proapoptotic effect, we measured the maximal increase in apoptotic index (E_{max}). The overall effect was measured as the sum of the E_{max} values of the antiproliferation and proapoptotic effects.

Immunohistochemistry

The data on Pgp, p53, and Bcl-2 expression in surgical specimens, before treatment with paclitaxel, were obtained from our previous study (42). aFGF and bFGF were detected by using previously described immunohistochemical methods (43). Briefly, after dewaxing and rehydration sequentially in xylene, ethanol, and water, tissue sections were boiled for 5 min in a 0.1 M citrate buffer (pH = 6.0), in a microwave oven, then cooled, washed in phosphate-buffered saline (PBS), and incubated with Dako blocking solution for 10 min and then with mouse antihuman aFGF antibody or bFGF antibody for 2 h in a humidified chamber at room temperature. The antibodies were diluted in PBS containing 5 mg/mL bovine serum albumin. The negative controls used mouse IgG as the primary antibody. The human ovarian cancer SKOV-3 cells, which contain high level of bFGF, were cultured as spheroid and then used as a positive control for normalizing interexperimental variation. Tissue sections were washed with PBS, covered with the linker solution and then peroxidase-conjugated streptavidin solution, again washed twice with PBS, and incubated for 5 min with diaminobenzidine and counterstained with hematoxylin. Protein expression was measured in a semiquantitative fashion by using a previously described

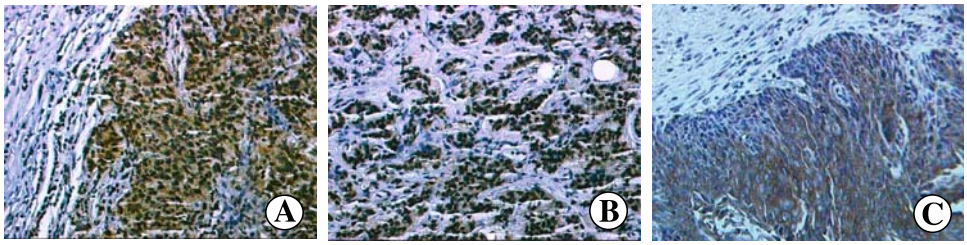

Fig. 1. Immunohistochemical detection of aFGF and bFGF in human tumors. (A) bFGF staining in a breast tumor. Note the staining in the cytoplasm and nuclei. (B) bFGF staining in a breast tumor. Note the localization in the nuclei. (C) aFGF staining in a head and neck tumor. Note the localization in the cytoplasm. Magnification ×400, chromogen: diaminobenzidine (brown), counterstained with hematoxylin (blue).

method (42). Briefly, this method employs a three-level grading system of the staining intensity (i.e., negative or −, positive or +0, and strong positive or ++), using different dilutions of the primary antibody. aFGF was stained with two antibody dilutions, 1:50 and 1:100. A score of − was given when a tissue failed to stain with either antibody dilutions, + for tissues stained positive only at the low antibody dilution, and ++ for tissues stained positive by both high and low dilutions. Similar criteria were used for bFGF, which was stained using 1:20 and 1:50 antibody dilutions. For each tumor, between 40 and 80 histocultures were processed for immunostaining.

Statistical and Bioinformatics Analysis

Differences in median values between multiple groups were analyzed the Kruskal–Wallis nonparametric test, followed by *post-hoc* evaluation of differences between individual groups using the Bonferroni correction. Softwares for statistical analysis (NPAR1WAY, and TTEST procedures) were obtained from SAS Inc. (Cary, NC, USA). Frequencies were compared by chi-square test (FREQ procedure). Predictive relationships between tumor pathologic parameters and tumor chemosensitivity were evaluated by linear regression analysis using the maximal r^2 selection method with the REG software routine of SAS. As an increase in model complexity or number of predictors usually increases the goodness-of-fit or r^2, we used the Akaike Information Criterion (AIC) to balance model simplicity and goodness of fit (44).

Our results indicated a positive correlation between bFGF and Pgp expression in patient tumors. To further evaluate the generality of this observation, we searched the cDNA Microarray Data of the NCI 60 Cancer Cell Lines (http://discover.nci.nih.gov/data setsNature2000.jsp) to analyze the relationship between *bFGF* and *mdr1* gene expressions.

RESULTS

FGF Expression in Human Tumors

Figure 1 shows the immunostaining of aFGF and bFGF in tumors. Table I summarizes the detection rates and staining intensity.

aFGF was detected in 49 of 96 tumors (51%). Bladder tumors showed the highest detection rate for aFGF (a total of 81% for tumors that showed + or ++ staining intensity), followed by prostate and ovarian tumors, whereas the lowest detection rates were found in head and neck, and breast tumors (<40%). aFGF staining was observed in the cytoplasm.

bFGF was detected in 61 of 96 tumors (63%). Ovarian tumors showed the highest detection rate (a total of 94% for

Table I. aFGF and bFGF Staining in Different Types of Tumors

	Number of tumors (% frequency)							
	aFGF				bFGF			
Tumor type	−	+	++	+ and ++	−	+	++	+ and ++
Bladder	3 (39)	7 (49)	6 (42)	13 (81)	7 (49)	5 (25)	4 (27)	9 (56)
Breast	11 (73)	4 (29)	0 (0)	4 (29)	6 (44)	7 (4)	2 (15)	9 (60)
Head/Neck	14 (64)	7 (26)	1 (6)	8 (42)	14 (64)	7 (26)	1 (3)	8 (34)
Ovarian	8 (4)	8 (4)	1 (7)	9 (53)	1 (7)	2 (12)	14 (82)	16 (94)
Prostate	11 (47)	9 (46)	6 (23)	15 (58)	7 (29)	12 (52)	7 (29)	19 (73)
Total	47 (54)	35 (34)	14 (17)	49 (13)	35 (42)	33 (35)	28 (31)	61 (63)

Protein expression was measured in a semiquantitative fashion, as described in Materials and Methods. aFGF was stained with two antibody dilutions, 1:50 and 1:100. A score of − was given when a tissue failed to stain with either antibody dilutions, + for tissues stained positive only at the low antibody dilution, and ++ for tissues stained positive by both high and low dilutions. Similar criteria were used for bFGF, which was stained using 1:20 and 1:50 antibody dilutions.

Table II. Relationship FGF and Other Tumor Pathobiological Parameters

	χ^2 (p value)				
Parameters	Stage	Grade	Pgp expression	p53 expression	Bcl-2 expression
aFGF	17.5 (<0.01)	2.9 (0.57)	0.2 (0.91)	2.9 (0.27)	3.1 (0.21)
bFGF	4.4 (0.35)	9.9 (0.04)	9.3 (0.01)	1.7 (0.43)	0.4 (0.81)

Expressions of proteins were graded by the immunostaning intensities (−, +, or ++, see Materials and Methods). The data were analyzed by chi-square test. A high chi square value together with a p value of less than 0.05 indicates a statistically significant correlation.

tumors that showed + or ++ staining intensity), followed by prostate, breast, and bladder tumors, whereas the lowest detection rates were found in head and neck tumors (<40%). The intracellular localization of bFGF varied among tumors; 37 tumors (60%) showed both cytoplasmic and nuclear staining, whereas primary staining in the cytoplasm or nucleus was observed in 12 (20%) and 12 (20%) tumors, respectively.

Relationship Between FGF Expression and Tumor Pathobiology

Table II shows the relationship between FGF expression, tumor pathobiologic parameters, and expression of p53 and Bcl-2 proteins. bFGF expression was positively correlated with Pgp expression and tumor grade ($p < 0.05$), but not with tumor stage or expression of p53 or Bcl-2 proteins. On the contrary, aFGF expression was negatively correlated with tumor stage ($p < 0.01$), but not with tumor grade or expression of Pgp, p53, or Bcl-2 proteins.

Linear regression analysis of the gene expression levels of *bFGF* and *mdr1* in the NCI 60 cell lines, as provided on the public database, indicated a statistically significant correlation ($p = 0.007$).

Table III. Pharmacological Effects of Paclitaxel in Human Tumors

	Antiproliferation effect		Maximal apoptotic index (%)	Overall maximal effect (%)
	E_{max} (%)	IC_{30} (μM)		
All tumors				
Range	0 to 100 ($n = 96$)	0.1 to >10 ($n = 96$)	0 to 29 ($n = 96$)	0 to 100 ($n = 96$)
Median	39	1.0	11	64
Mean ± SD	40 ± 24	4.0 ± 4.6	12 ± 7	60 ± 22
Responding tumors				
Range	14 to 100 ($n = 83$)	0 to >10 ($n = 83$)	1.5 to 36 ($n = 92$)	16 to 100 ($n = 92$)
Median	45	0.5	12	62 ± 20
Mean ± SD	46 ± 19	3.1 ± 4.2	12 ± 7	65

The pharmacodynamic data of the antiproliferative and proapoptotic effects of paclitaxel were obtained from our previous studies (42). For the antiproliferation effect, the maximal effect (E_{max}) and the paclitaxel concentration that produced 30% inhibition of DNA precursor incorporation (IC_{30}) are provided. The proapoptotic effect is presented as the maximal increase in apoptotic index. The overall effect is the maximal inhibition of DNA precursor-labeling index in the remaining viable or nonapoptotic cells.

Effects of Paclitaxel on Tumors

Three aspects of paclitaxel effects, i.e., inhibition of DNA synthesis, induction of apoptosis, and the sum of both effects (overall effect), were evaluated. Table III summarizes the results.

For the antiproliferation effect, paclitaxel inhibited DNA synthesis in 86% (83/96) of tumors, with no effect (i.e., not significantly different from 0% inhibition) on the remaining 13 tumors. Figure 2 shows a representative sigmoidal relationship between paclitaxel concentration and inhibition of DNA synthesis, in a head and neck tumor. With the exception of one tumor that showed a 100% response, the remaining tumors showed less-than-complete response, where the maximal effect (E_{max}) was achieved at 1 μM drug concentration and was not enhanced when the drug concentration was increased to 10 μM. The average E_{max} in the 83 responding tumors was 46%. The paclitaxel concentration that produced 30% inhibition (IC_{30}) showed a large, >10,000-fold range, with the average value of 1 μM As the maximal effect was less than 50% in most tumors, the IC_{30} and E_{max} values were used to compare the relative sensitivity of individual tumors to paclitaxel.

For the proapoptotic effect, 86% (83 of 96) of untreated tumors showed <1% apoptotic index, whereas the remaining 14% showed an index between 1 and 5.5%. Paclitaxel treatment significantly increased the apoptotic index in 96%

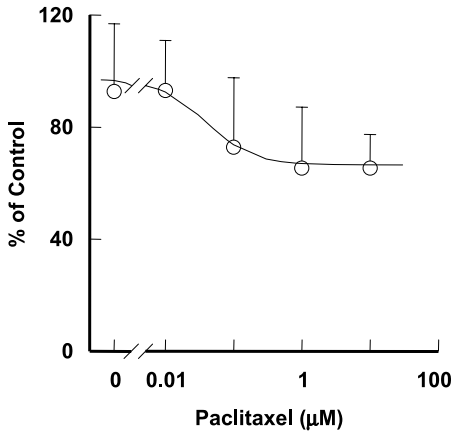

Fig. 2. Relationship between paclitaxel concentration and inhibition of DNA synthesis. A representative concentration–effect relationship in a head and neck tumor is shown. The maximal inhibition was less than 50%. Mean ± SD.

(92 of 96) of the tumors, with no effect in the remaining tumors. The average increase in apoptotic index in the 92 responding tumors was 13%.

The overall maximal paclitaxel cytotoxicity or the combination of the maximal antiproliferation and pro-apoptotic effects, observed in 92 tumors, was about 60%.

Relationship between FGF expression and paclitaxel effects

We analyzed the relationships between paclitaxel effects and aFGF/bFGF levels, as well as tumor pathobiological parameters (stage, grade, labeling index, and expression of p53

Table IV. Correlation Between Pathobiological Parameters and Maximum Paclitaxel Activity

Parameters	r^2	AIC	p	Correlation
Inhibition of DNA synthesis (maximal inhibition of proliferation index) of paclitaxel				
bFGF	0.16	595	<0.01	Negative
Stage	0.14	596	<0.01	Negative
Pgp	0.11	600	<0.01	Negative
Proliferation or labeling index (LI)	0.08	603	<0.01	Negative
Grade	0.07	604	0.01	Negative
p53	0.06	605	0.02	Negative
Bcl-2	0.05	606	0.03	Negative
aFGF	0.03	608	0.1	NA
bFGF + Stage	0.26	584	<0.01	NA
bFGF + Stage + Bcl-2	0.30	581	<0.01	NA
bFGF + Stage + Bcl-2 + p53	0.33	579	<0.01	NA
bFGF + Stage + Bcl-2 + p53 + aFGF	0.34	579	<0.01	NA
bFGF + Stage + Bcl-2 + p53 + aFGF + Pgp	0.36	579	<0.01	NA
bFGF + Stage + Bcl-2 + p53 + aFGF + Pgp + LI	0.36	580	<0.01	NA
bFGF + Stage + Bcl-2 + p53 + aFGF + Pgp + LI + Grade	0.36	582	<0.01	NA
Proapoptotic effect (maximal increase in apoptotic index) of paclitaxel				
Pgp	0.29	352	<0.01	Positive
LI	0.28	354	<0.01	Positive
Grade	0.09	376	<0.01	Positive
bFGF	0.03	383	0.13	NA
aFGF	0.02	383	0.16	NA
Stage	0.02	384	0.22	NA
Bcl-2	0.005	385	0.50	NA
p53	0.001	385	0.80	NA
Pgp + LI	0.41	336	<0.01	NA
Pgp + LI + aFGF	0.44	333	<0.01	NA
Pgp + LI + aFGF + p53	0.45	334	<0.01	NA
Pgp + LI + aFGF + p53 + Grade	0.46	335	<0.01	NA
Pgp + LI + aFGF + p53 + Grade + bFGF	0.46	336	<0.01	NA
Pgp + LI + aFGF + p53 + Grade + bFGF + Stage	0.46	338	<0.01	NA
Pgp + LI + aFGF + p53 + Grade + bFGF + Stage + Bcl2	0.46	339	<0.01	NA
Overall effect (sum of maximal E_{max} of antiproliferation and apoptosis) of paclitaxel				
bFGF	0.12	587	<0.01	Negative
LI	0.11	588	<0.01	Negative
Stage	0.10	589	<0.01	Negative
p53	0.08	591	<0.01	Negative
Bcl-2	0.03	596	0.10	NA
Pgp	0.03	596	0.10	NA
aFGF	0.03	597	0.11	NA
Grade	0.03	597	0.11	NA
bFGF + LI	0.20	580	<0.01	NA
bFGF + LI + aFGF	0.24	577	<0.01	NA
bFGF + LI + aFGF + p53	0.28	574	<0.01	NA
bFGF + LI + aFGF + p53 +Bcl-2	0.30	573	<0.01	NA
bFGF + LI + aFGF + p53 +Bcl-2 + Stage	0.31	574	<0.01	NA
bFGF + LI + aFGF + p53 +Bcl-2 + Stage + Pgp	0.31	575	<0.01	NA
bFGF + LI + aFGF + p53 +Bcl-2 + Stage + Pgp + Grade	0.31	577	<0.01	NA

The correlation between FGF expression and other pathobiological parameters and the maximal effects of paclitaxel in three categories (i.e., inhibition of DNA synthesis, apoptosis, overall effect) were analyzed using linear regression (maximal r^2 selection method). Higher r^2 and lower AIC values indicate a better correlation. For the combination of two and more parameters, only those that show the highest r^2 were listed. A negative correlation indicates a lower paclitaxel effect at a higher value of the parameter. A positive correlation indicates a higher paclitaxel effect at a higher parameter value. NA, not applicable.

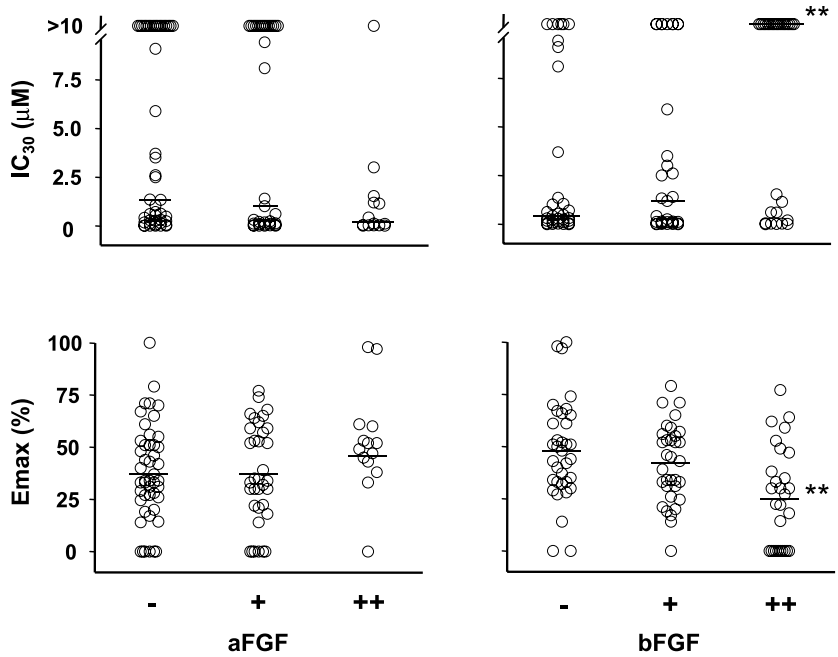

Fig. 3. Relationship between FGF expression in surgical specimens of patient tumors and antiproliferation effect of paclitaxel. (a) IC_{30} *vs.* aFGF expression. (b) IC_{30} *vs.* bFGF expression. (c) E_{max} *vs.* aFGF expression. (d) E_{max} *vs.* bFGF expression. Values for individual tumors are shown. Tumors are grouped by the extents of FGF expression (− or undetectable, + or low, ++ or high), as defined in Materials and Methods. The horizontal bars indicate the median values of IC_{30} or E_{max}. No significant differences in the IC_{30} or E_{max} values were found for tumors with different aFGF expression. In contrast, tumors expressing high bFGF levels showed significantly higher IC_{30} and lower E_{max} values compared to tumors with lower bFGF levels (**$p < 0.01$).

and Bcl-2), using multivariate regression analysis. The most significant correlations for two to eight parameters, for paclitaxel effects, are detailed in Table IV.

For inhibition of DNA synthesis, as neither IC_{30} nor E_{max} was normally distributed, we used their median values to evaluate the relationships of these parameters with FGF expressions. Figure 3 shows the results. The respective median values of IC_{30} for tumors expressing undetectable, low and high protein levels were 1.35, 1.00, and 0.21 μM for aFGF, and 0.43, 1.19, and 10.0 μM for bFGF. The respective median values of E_{max} for these tumor groups were 37, 37, and 46% for aFGF, and 48, 42, and 25% for bFGF. No significant differences in the median IC_{30} or E_{max} values were found for tumors with different aFGF expression ($p = 0.103$ for IC_{30} and $p = 0.147$ for E_{max}, Kruskal–Wallis test with *post-hoc* analysis). In contrast, tumors expressing high bFGF levels showed significantly higher IC_{30} and lower E_{max} values compared to tumors with no or low bFGF levels ($p = 0.036$ for IC_{30} and $p = 0.001$ for E_{max}). Conversely, tumors that did not show detectable bFGF level showed the lowest IC_{30} and highest E_{max} values. These results suggest a correlation between high bFGF expression and low drug sensitivity.

Further analysis indicated that in addition to bFGF, six pathobiologic parameters (i.e., tumor stage, tumor grade, labeling index, p53 status and expression of Pgp and Bcl-2 proteins) showed significant correlations with the antiproliferation effect of paclitaxel. As with bFGF, all correlations were negative (i.e., higher values in each parameter correlated with lower E_{max} values, or resistance). As a single parameter, bFGF expression showed the best correlation with resistance. Inclusion of other parameters further improved the relationships.

For the proapoptotic effect of paclitaxel, only three parameters (tumor grade, labeling index, and Pgp expression) showed significant correlations. Opposite to the correlations with the antiproliferation effect of paclitaxel, all correlations with the proapoptotic effect were positive (i.e., higher values in each parameter correlated with higher E_{max} values, or sensitivity). bFGF and aFGF expression showed a trend of positive correlation, but neither relationship reached statistical significance.

For the overall drug effect, four parameters (tumor stage, labeling index, expression of bFGF, and p53 status) showed significant correlations with resistance; the best correlation was observed with bFGF expression.

A comparison of the intracellular localization of bFGF (i.e., cytoplasm, nuclei, or both) with paclitaxel effects did not show significant correlation (not shown).

DISCUSSION

The present study evaluated 96 patient tumors derived from five tumor types (bladder, breast, head and neck, ovarian, prostate) where paclitaxel has shown clinical activity. Results obtained using these tumors maintained in the

three-dimensional histoculture system indicate bFGF as the most important predictor of paclitaxel resistance, compared to other pathobiological factors including tumor grades, stages, and expressions of MDR, p53, bcl-2, and aFGF. The aFGF level did not correlate with paclitaxel activity. It is noted that aFGF plus bFGF was the second leading indicator for the resistance of paclitaxel-induced antiproliferative effect after bFGF plus tumor stages in the two-parameter analysis. The correlations between paclitaxel activity and tumor pathobiological parameters other than the FGFs have been discussed in an earlier article (35).

The present study used a semiquantitative, immunohistochemical method to evaluate the FGF levels in the tumors. This is a widely used method and, as reported here, has yielded valuable information. For future studies of the relationship between FGF inhibition and chemosensitization, as would be needed to identify the optimal pharmacodynamics of FGF inhibitors, a more quantitative method such as image analysis is needed to translate the immunohistochemical staining intensity to actual protein levels.

An additional interesting and unexpected finding of the present study is the positive correlation between bFGF and Pgp protein levels in human tumors and the correlation between *bFGF* and *mdr1* gene expressions in the NCI 60 human cancer cell lines. We previously showed that high Pgp protein levels correlated with a greater extent of paclitaxel-induced apoptosis in human tumors (42). This finding was subsequently confirmed in MCF7 breast cancer cells transfected with *mdr1*; this Pgp effect seems to be specific to antimicrotubule agents and is unrelated to the drug efflux function of Pgp (45,46). bFGF can initiate multiple signal transduction pathways including the phosphatidylinositol 3 kinase–Akt 1 pathway, Ras–MAP kinase pathway, and protein kinase C (PKC)-dependent signal transduction pathway (47,48). PKC, in turn, can phosphorylate Pgp, the *mdr1* product. PKC is also often activated in cell lines demonstrating multidrug resistance (49,50). Furthermore, both *mdr1* and *bFGF* promoters are activated by mutant p53 and repressed by wild-type p53 (51–55). These various commonalities suggest a possible relationship between the broad-spectrum resistance conferred by *mdr1* and bFGF.

In summary, the results of the present study indicate bFGF as the most important prognostic indicator of paclitaxel resistance in human patient tumors, which is consistent with our earlier findings in preclinical models, and suggest bFGF as a potentially important chemoresistance mechanism in human solid tumors, maintained as three-dimensional cultures.

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