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Basic Fibroblast Growth Factor and Ovarian Cancer

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The factor(s) which regulate the rapid growth of ovarian epithelial carcinoma, as well as other types of malignant tumors, are still largely unknown. Recently, experimental evidence indicated that neoplastic cells are able to synthesize peptide growth factor and their receptors. This autocrine secretion could be one of the mechanisms to sustain their abnormal proliferation. In this study, we evaluated the possible role of basic fibroblast growth factor (bFGF) that is a likely candidate because it has both angiogenic and mitogenic activity and has been found in a variety of other neoplasms. As assessed by both bioassay and radioimmunoassay, a bFGF-like protein was present in seven ovarian epithelial neoplasms and in primary culture of dispersed ovarian cancer cells. Levels of this protein as well as its bioactivity varied in the different tumors examined. Reverse transcription-polymerase chain reaction indicated that the genes for bFGF and its receptor are expressed in all the samples studied. These data suggest that bFGF might be one of the growth factor regulating ovarian cancer cell proliferation through an autocrine mechanism. We are currently investigating whether the expression of this growth factor varies as a function of the histologic grade of the tumors.

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In females, ovarian cancer accounts for 4% of cancers and 6% of cancer deaths [1]. The vast majority of this female reproductive cancer is of epithelial and not of stromal origin. Epidemiological studies have shown that steroid hormones involved in menstrual activity might play a role in the pathogenesis of ovarian cancer [2]. Estrogen and progesterone receptors are found in about half of the ovarian cancers and the presence of both types of receptors is seen in about one third of the cases [2]. Different studies indicate that histologic grade, stage of the disease, ploidy and age of the patient can influence disease free survival. However, the 5 y survival rate is still very low [3] and new approaches are needed to improve this poor prognosis.

In recent years, much attention has been focused on the role of growth factors and the activation of oncogenes in the development of human malignancies. There is increasing evidence that tumor-derived growth factors are important in the proliferation of malignant cells [4–6]. Inappropriate production of growth factors along with coexpression of growth factor receptors can result in autocrine stimulation.

As a component of studies designed to further investigate the pathogenetic mechanisms of human ovarian cancer, we evaluated the possible role of basic fibroblast growth factor (bFGF). This peptide growth factor is a likely candidate because it has both mitogenic and angiogenic properties [7], has been found in a variety of other neoplasms [8–12] and is synthesized in normal ovarian cells [13–15]. Thus, we set up experiments to determine whether bFGF is present in and is synthesized by human ovarian epithelial neoplasms. Moreover, we also examined the expression of the gene for the bFGF receptor.

To investigate whether bFGF is present in human ovarian neoplasms, heparin-sepharose affinity chromatography was performed on extracts of these tissues homogenized in 0.5% Triton X-100 as previously described [16]. The column was sequentially eluted with 10 mM Tris-HCl containing 0.6 1.0 or 3.0 M NaCl. The eluted fractions were analyzed for their ability to stimulate the proliferation of bovine adrenal cortical endothelial (ACE) cells in a standard bioassay for bFGF [16]. Figure 1 shows that elution of the column with 3 M NaCl yielded a major peak of bioactivity. The same results were obtained when the tumor cells were dispersed [16] and their cytoplasmic extract subjected to heparin-sepharose chromatography

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(Fig. 2). This chromatographic profile is similar to that of bFGF which is eluted with the highest salt concentration.

The chromatographic fractions positive for the presence of mitogenic activity also showed bFGF-like immunoreactivity when analyzed by an RIA that does not cross-react with acidic FGF and other related peptides [16] (Fig. 1). The amount of bFGF immunoreactivity present in the positive fractions of all the tumors studied correlated well with the results obtained by bioassay (Table 1). Finally, the identity of the mitogenic substance present in the ovarian tumors and bFGF was further demonstrated using a polyclonal antibody. When ACE cells were cultured in the presence of an aliquot of purified tumor extract with an anti-bFGF antibody, the mitogenic effect previously observed was completely abolished (Fig. 3). To assess whether the presence of the protein in the ovarian tumors was due to *in situ* synthesis, we evaluated the expression of bFGF mRNA. This was accomplished by amplifying bFGF mRNA target sequences using polymerase chain reaction according to a protocol previously described [16]. The primer sequences used have been successfully employed to detect bFGF transcript in rat ovaries [14]. They are specific for regions separated by the first intron of the bFGF gene [17] so that the amplified fragments could only have originated from mRNA and not from genomic DNA.

Figure 4 shows that reverse transcription of ovarian tumor mRNA generated a DNA product corresponding to the predicted length, 354 bp, of the bFGF amplification product. Finally, to evaluate whether

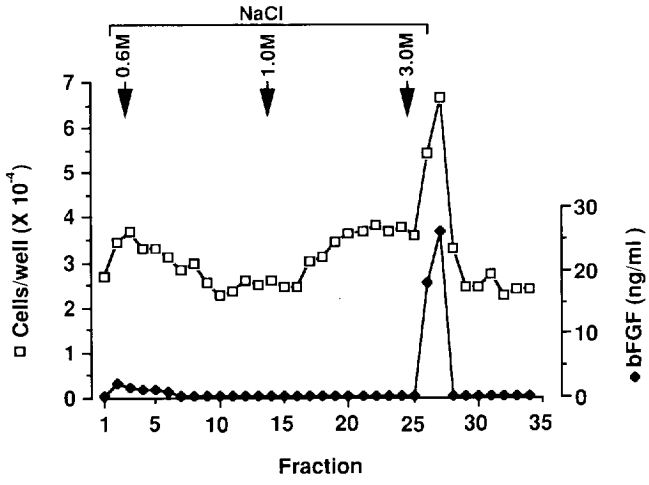

Fig. 1. Heparin-sepharose affinity chromatography of crude extract of human ovarian neoplasm. Tumor extract was applied to heparin-sepharose affinity column and eluted as described in text. Ten μl aliquots of the chromatographic fractions were added every other day to bovine adrenocortical endothelial cells seeded at a density of 5×10^3 in a 16 mm/well. Cell proliferation (□) was determined after 5 days. All fractions were also analyzed in a radioimmunoassay specific for bFGF (♦) Values represent means of duplicate determinations, which varied by less than 10%.

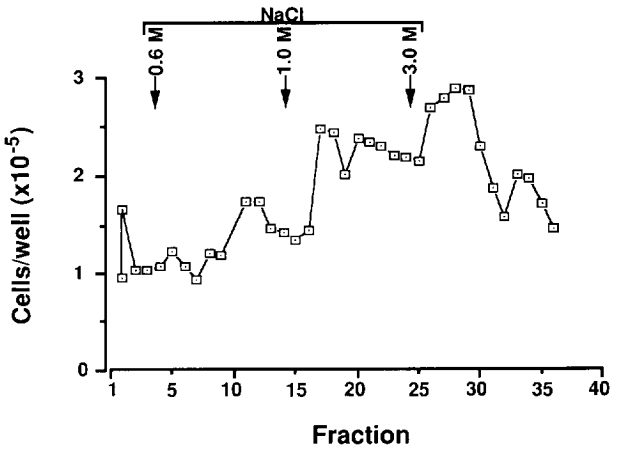

Fig. 2. Heparin-sepharose affinity chromatography of cytoplasmic extract derived from human ovarian tumor cells in primary culture. Tumor cells were dispersed and cultured as described in text. The cytoplasmic extract was then purified by heparin-sepharose affinity column and bioassayed using bovine adrenocortical endothelial cells. Experimental conditions are the same as those in Fig. 1.

bFGF might act through an autocrine mechanism, we also determined if bFGF receptor gene was expressed in these neoplastic tissues. As shown in Fig. 5 using specific oligonucleotide primers, a 661 bp DNA fragment of the human bFGF receptor sequence was amplified in all the samples studied. Taken together, the experiments presented herein strongly suggest that human ovarian epithelial neoplasms contain a biologically active mitogenic substance indistinguishable by bFGF. This is demonstrated evaluating different parameters: its chromatographic profile, its immunoreactivity, its mitogenic action on bFGF target cells. Moreover, reverse transcription-polymerase chain reaction (RT-PCR) indicates that the presence of this protein is most likely due to *in situ* synthesis as the

Table 1. bFGF-like bioactivity and immunoreactivity in human ovarian epithelial neoplasms

Patient	Fraction	Bioassay (% Stimulation)*	RIA (ng/ml)
G.M.	30	35	4
A.C.	26	100	18
	27	153	26
R.M.	28	69	8
	29	87	10
	30	53	6
A.T.	27	74	12
	28	90	33
G.M.	27	38	10
	28	87	12
G.D.	28	30	10
	29	42	15

*Increase in target cell proliferation expressed as percentage of control cultures.

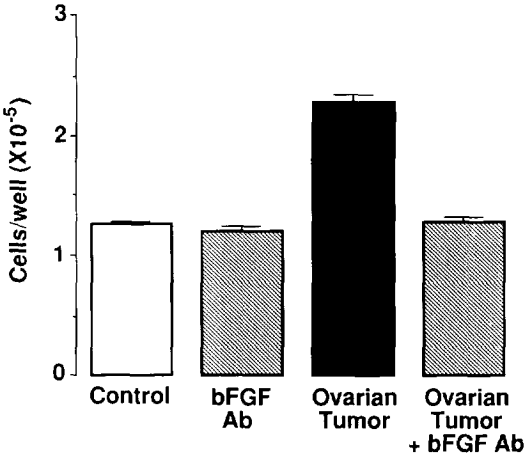

Fig. 3. Effect of anti-bFGF antibody on proliferation of adrenocortical endothelial cells exposed to heparin-sepharose purified ovarian tumor extract. Cells were seeded at a density of 5×10^3 cells/16 mm well in the absence or presence of purified ovarian tumor extract added every other day. Anti-bFGF antibody was also added every other day. Cells were counted after 5 days. Values are expressed as mean ± SEM of triplicate determinations.

bFGF gene is expressed in all the tumors studied. Finally, the expression of the bFGF receptor gene in the same samples allows speculation that bFGF might act through an autocrine mechanism.

bFGF has both mitogenic and angiogenic properties and therefore it has been implicated as a potential regulator of cell growth in several human tumors including melanoma [8], bladder and kidney carcinoma [11], rabdomiosarcoma [9] and gliomas [10]. Modulation of bFGF synthesis by antisense oligonucleotides inhibits the growth of transformed human astrocytes, although it does not affect the growth potential of normal astrocytes [10].

The results of the present studies are consonant with other data supporting a role for the autocrine synthesis of growth factors in the development of human ovarian neoplasms. Indeed, it has been previously reported that epidermal growth factors and its receptor are present in ovarian carcinoma and their presence seems to be correlated with a poor prognosis [18–20]. Furthermore, ascitic fluid from ovarian cancer patients does contain mitogenic activity from at least one unique growth factor [21, 22]. Our data indicate that ovarian cancer cells are able to synthesize bFGF and its receptor but do not elucidate yet the mechanisms by which bFGF could play a role in their abnormal proliferation. We are currently investigating whether quantitative differences in bFGF synthesis might be present in the different tumors studied. To this aim, we are using a competitive RT-PCR employing an internal standard to obtain semiquantitative results and thus compare the

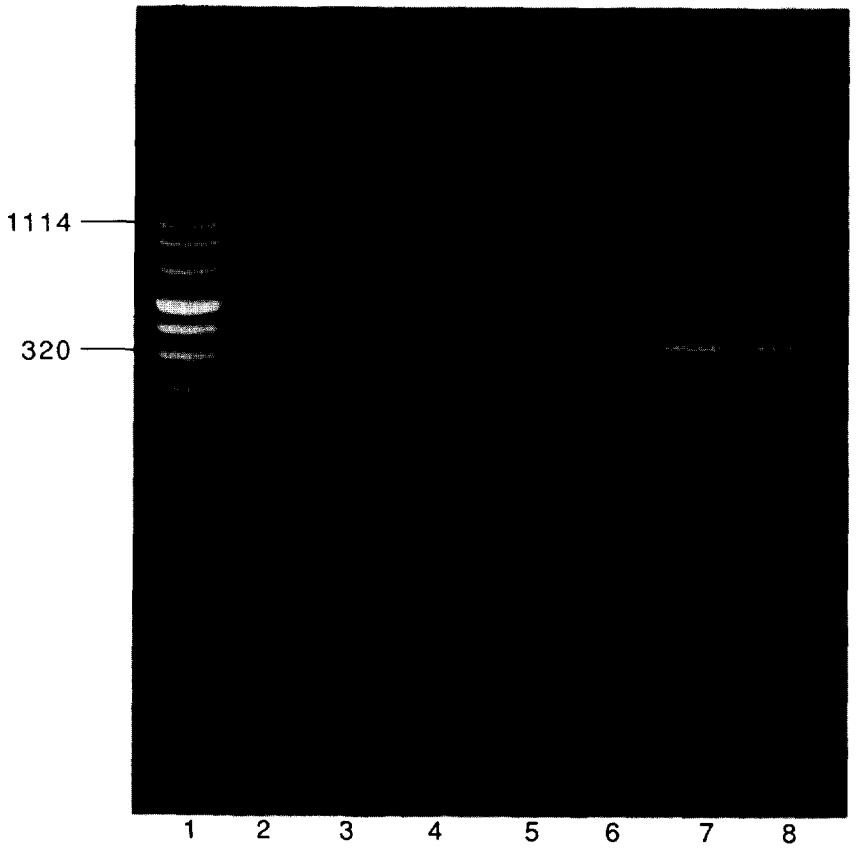

Fig. 4. Analysis of bFGF mRNA by polymerase chain reaction. One μg of total RNA from seven human ovarian tumors (lanes 2–8) was reverse transcribed and amplified using primers specific for bFGF. 10% of the PCR mixture was resolved on a 4% agarose gel stained with ethidium bromide. Lane 1, size markers. bFGF primers amplified a 354 bp fragment.

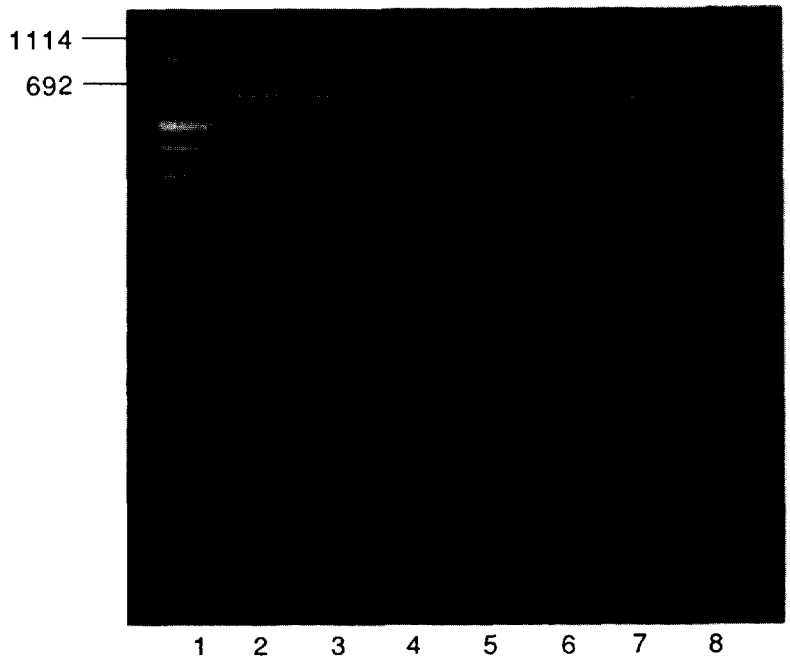

Fig. 5. Analysis of bFGF receptor mRNA by PCR. One μg of total RNA from the same samples of Fig. 4 (lanes 2–8) was reverse transcribed and amplified. 10% of the PCR mixture was resolved on a 4% agarose gel stained with ethidium bromide. Lane 1, size markers. bFGF receptor primers amplified a 661 bp fragment.

levels of bFGF synthesis in different samples. However, whether overexpression of bFGF might be a function of the histological differences between tumors needs to be established on a larger number of cases. Moreover, we are currently investigating whether unique bFGF isoforms with enhanced mitogenic activity might be present in human ovarian neoplasms.

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