Vol. 174, No. 1, 1991 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
January 15, 1991 Pages 136-141

UP-REGULATION OF FIBROBLAST GROWTH FACTOR (FGF) RECEPTOR mRNA LEVELS BY BASIC FGF OR TESTOSTERONE IN ANDROGEN-SENSITIVE MOUSE MAMMARY TUMOR CELLS

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Received November 5, 1990

Since we had previously shown that both basic fibroblast growth factor (bFGF) and testosterone stimulate the growth of mouse mammary carcinoma cells (SC-3) in serum-free culture, we tested the effect of bFGF or testosterone on FGF receptor mRNA levels. Northern blot analyses revealed that stimulation with bFGF resulted in a 5-fold increase in FGF receptor mRNA levels at 6-8 h followed by a decline to the unstimulated levels at 24 h. Simultaneous addition of cycloheximide blocked bFGF-induced accumulation of FGF receptor mRNA, although exposure of SC-3 cells to cycloheximide alone caused marginal increase in its basal level. Neither phorbol ester nor forskolin stimulated FGF receptor mRNA expression, but testosterone could raise FGF receptor mRNA levels. To obtain the maximum stimulation, however, testosterone required the longer stimulation period (12 h) than bFGF, suggesting that testosterone-induced FGF receptor mRNA accumulation is mediated through an induction of FGF-like growth factor.

Fibroblast growth factor (FGF) has been well known to have the mitotic effects on many types of cells (1). However, its mode of aciton remains largely obscure. To clarify this important but undissolved issue, the extensive studies on the regulatory mechanisms of FGF receptor (FGF-R) expression and function are definitely required. Recent cloning tasks of chick (2), mouse(3) or human (4) FGF-R cDNA successfully clarified the molecular nature of FGF-R. To our knowledge, however, the regulatory mechanism of FGF-R mRNA expression is not yet reported. In this relation, the expression of some cell-surfaced receptor mRNAs has been reported to be down-

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Abbreviations: FGF, fibroblast growth factor; FGF-R, fibroblast growth factor receptor; T, testosterone; MEM, Eagle's minimum essential medium; HMB medium, Ham F-12: MEM (1:1, v/v) containing 0.1 % bovine serum albumin; SSC, 0.15 M NaCl, 15 mM sodium citrate; Denhardt's solution, 0.02 % (w/v) Ficoll, 0.02 % (w/v) polyvinyl pyrrolidone, 0.02 % (w/v) bovine serum albumin; SDS, sodium dodecyl sulfate; TPA, 12-O-tetradecanoylphorbol 13-acetate; EGF, epidermal growth factor; EGF-R, EGF receptor.

0006-291X/91 $1.50

regulated (5) or up-regulated (6) by their corresponding ligand. This ligand-dependent alteration in receptor mRNA levels seems to be important because this may be related to "shut-off" or "amplification" of ligand stimuli.

Mouse mammary carcinoma cells (SC-3 cells) cloned from Shionogi carcinoma 115 have been observed to be growth-stimulated by testosterone (T) under a serum-free condition (7). This growth enhancement is mediated through an androgen-induced FGF-like growth factor which is able to be associated with FGF-R (8). In addition, the growth of SC-3 cells is enhanced by authentic FGFs (9). These observations prompted us to examine the effects of FGF or T on FGF-R mRNA levels using SC-3 cells.

MATERIALS AND METHODS

SC-3 cells were cloned and maintained as described previously (7). These cells were plated at a density of 10^6 cells/100-mm dish in Eagle's minimum essential medium (MEM) supplemented with 2 % fetal calf serum (FCS). Two days later, the cells were washed with Dulbecco's phosphate-buffered saline and then cultured for 24 h in HMB medium. To completely eliminate the residual FCS effects, HMB medium was changed once more. After 12 h, SC-3 cells were stimulated with various test compounds at the concentrations indicated in each figure legend. After stimulation for the various periods of time, SC-3 cells were lysed in 5.5 M guanidium isothiocyanate and RNA was precipitated through 5.7 M CsCl as described before (10). The total RNA (15 μg/lane) was electrophoresed in 1 % agarose gel containing 0.66 M formaldehyde and transferred onto a nitrocellulose filter. Sal I-Apa I fragment (0.4 kb) encoding the region from the juxtamembrane to kinase domain of chick FGF receptor, which was kindly supplied by Dr. L.T. Williams (2), was employed as a probe after being labeled with [^{32}P]dCTP by a random priming method. Hybridization was then carried out in 50 % (v/v) formamide; 5xSSC ; 20 mM sodium phosphate,pH 7.4 at 20 °C; 5xDenhardt's solution ; 0.1 % (w/v) SDS; 10 % (w/v) dextran sulfate, 100 g/ml denatured salmon sperm DNA at 42 °C for 18 h. Blots were subsequently washed twice with 2xSSC, 0.1 % SDS at room temperature and three times with 0.3xSSC, 0.1 % SDS at 50 °C for 30 min. Autoradiography was carried out at -70 °C with Kodak X-Omat AR film with intensifying screens. The filters were rehybridized with [^{32}P]-labeled β-actin cDNA as described above. The semiquantification of band intensities was performed by densitometric scanning.

The chemicals used here were obtained from the following sources: bovine basic FGF (bFGF) from R&D systems, Inc. (Minneapolis, MN); β-actin cDNA from Wako Pure Chemical Industries, Ltd. (Osaka, Japan); the restriction enzymes from Toyobo Co., Ltd., (Tokyo, Japan); forskolin (7-0-hemisuccinyl-7-deacetyl form) from Calbiochem-Behring Corp., American Hoechst Corp., (San Diego, CA); 12-0-tetradecanoylphorbol 13-acetate (TPA), cycloheximide and T from Sigma Chemical Corp., (St Luis, Mo). The other reagents used here were of analytical grade.

RESULTS

Northern blot analyses showed [^{32}P]-labeled FGF-R cDNA was hybridized with RNA at the region of approximately 3.5 kb, which is consistent with the

size of FGF-R mRNA in mouse 3T3 cells (3)(Fig. 1A). The stimulation of SC-3 cells with bFGF (2 ng/ml) resulted in the accumulation of FGF-R mRNA. The maximal accumulation was observed at a 6-8 h stimulation, followed by a decline to a near control level at 24 h. The semiquantification by a densitometric analysis revealed a 5-fold increase in FGF-R mRNA levels at 6-8 h (Fig.1B). The same filter was rehybridized with [^{32}P]-labeled β-actin cDNA, showing a slight increase in β-actin mRNA levels at 8-24 h stimulation, although ethidium bromide staining confirmed that the same amount of RNA was applied to each lane (data not shown).

When SC-3 cells were stimulated with various concentrations of bFGF for 6 h, a concentration-dependent increase in FGF-R mRNA reaching to maximal level at 0.1 ng/ml was observed. The half-maximal effect of bFGF on FGF-R mRNA accumulation was obtained at 0.005-0.01 ng of bFGF/ml (Fig. 2). To further delineate the mechanism of a ligand-dependent accumulation of FGF-R mRNA, the effects of various reagents modulating the protein synthesis or phosphorylation were examined (Fig. 3). When cycloheximide, the protein synthesis inhibitor, was added to SC-3 cells at a concentration of 10 μg/ml, FGF-R mRNA was increased about 3-fold. However, the simultaneous addition

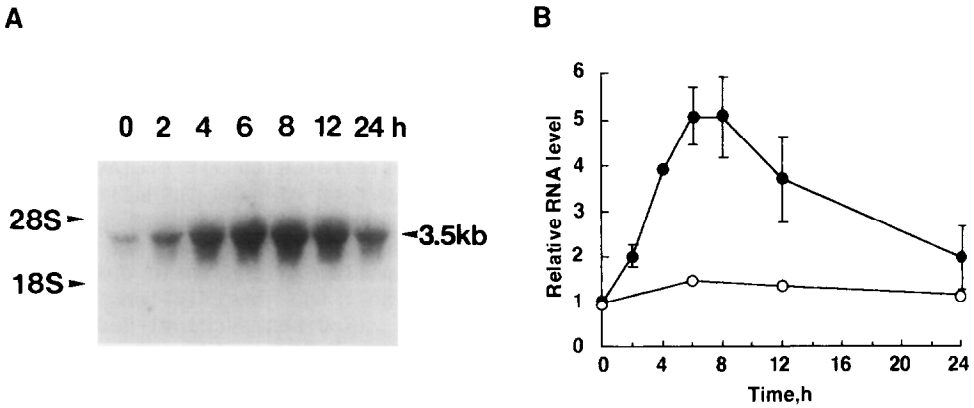

Fig. 1 Accumulation of FGF-R mRNA in response to bFGF stimuli
SC-3 cells were stimulated with 2 ng/ml bFGF for the indicated periods of time. Total RNA (15 μg/lane) was electrophoresed and hybridized with [^{32}P]-labeled chick FGF-R cDNA. Autoradiography was then obtained by an exposure to X-Omat AR film at -70°C for 2 days (Panel A). Band intensities were semiquantified by densitometric analyses and expressed as a fold-increase (●). Non-stimulated cells were also analyzed (○) (Panel B). The values (means ± S.E.) on stimulated cells, were obtained by three separate experiments (with the exception of the 4-hr point).

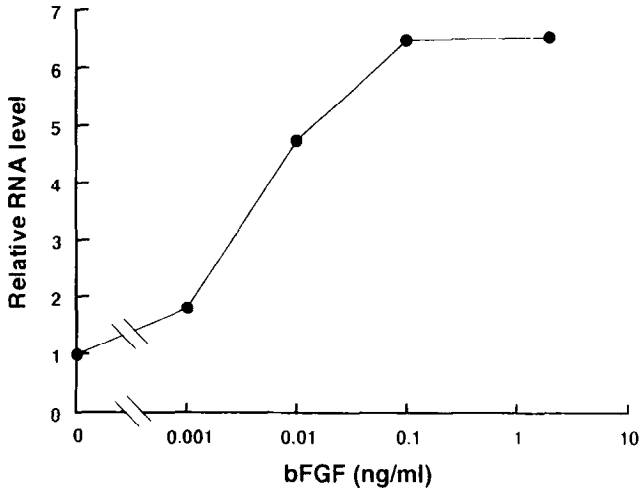

Fig. 2 Effect of bFGF concentrations on FGF-R mRNA levels
SC-3 cells were stimulated for 6 h with the indicated concentrations of bFGF. FGF-R mRNA levels were analyzed as described in the legend of Fig. 1.

of cycloheximide with bFGF (2 ng/ml) failed to further elevate FGF-R mRNA level. In addition, TPA or forskolin, when it is added alone or in combination with bFGF, did not show the significant effect on FGF-R mRNA levels, suggesting that neither protein kinase C nor A plays a role in regulation of FGF-R mRNA levels.

Since SC-3 cells have been observed to be androgen-sensitive and to be growth-stimulated by androgen-induced FGF-like growth factor (7), the effect of androgen on FGF-R mRNA levels was addressed. As shown in Fig. 4, T stimulation caused a gradual accumulation of FGF-R mRNA. Compared with bFGF

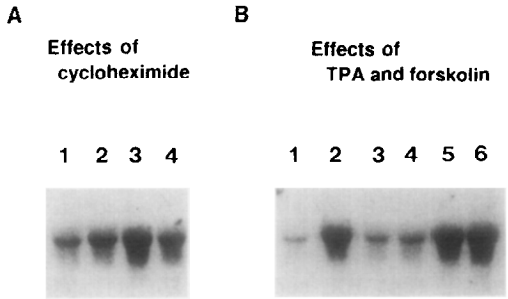

Fig. 3 Effect of various reagents on FGF-R mRNA levels
SC-3 cells were exposed to cycloheximide (10 μg/ml), TPA (100 nM) or forskolin (25 μM) for 6 h. The effects of these reagents on bFGF-induced accumulation of FGF-R mRNA were also studied.
Panel A: lane 1, no addition; lane 2, cycloheximide; lane 3, bFGF; lane 4, cycloheximide and bFGF.
Panel B: lane 1, no addition; lane 2, bFGF; lane 3, forskolin; lane 4, TPA; lane 5, forskolin and bFGF; lane 6, TPA and bFGF.

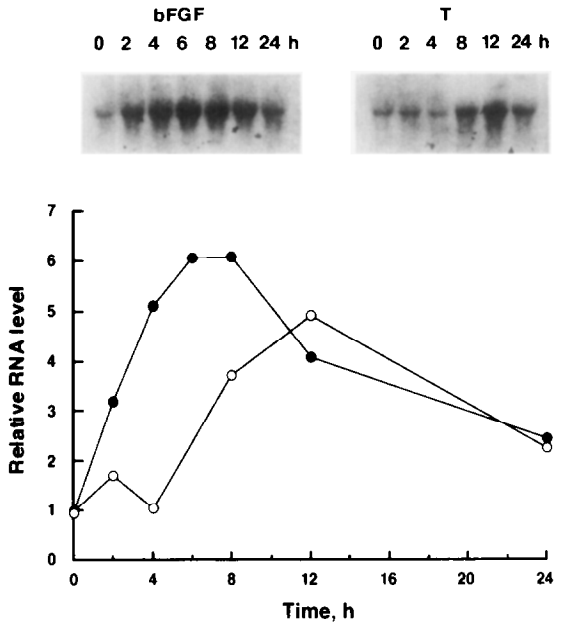

Fig. 4 Time course of bFGF- or T-induced accumulation of FGF-R mRNA
SC-3 cells were stimulated with 10 nM T (○) or 2 ng/ml (●) bFGF for the indicated periods of time. FGF-R mRNA levels were then analyzed as described above.

stimulation, a slightly longer period (~12 h) was required to obtain the maximum stimulation with T.

DISCUSSION

We have shown that exposure of SC-3 cells to bFGF or T stimulates accumulation of FGF-R mRNA. To carry out these experiments, a portion of chick FGF-R cDNA was employed as [^{32}P]-labeled probe. Recent cloning study on FGF-R cDNA of SC-3 cells in our laboratory revealed that the nucleotide sequence of this probe has a 85 % homology with FGF-R cDNA of SC-3 cells (Kouhara: manuscript in preparation). The addition of cycloheximide to SC-3 cells stimulated FGF-R mRNA accumulation, suggesting that rapid-turnover protein(s) negatively regulate FGF-R mRNA levels in SC-3 cells. This result is similar to the finding that EGF receptor (EGF-R) mRNA contents are elevated by exposure of KB cells to cycloheximide (6). In contrast with the result on EGF-R mRNA, bFGF failed to further enhance FGF-R mRNA levels in cycloheximide-treated SC-3 cells. This result indicates that a ligand-dependent elevation of FGF-R mRNA levels is also positively regulated by a protein whose synthesis

is under a control of bFGF. In addition, protein kinase C activation with TPA does not affect FGF-R mRNA levels, which is also distinct from the observation on EGF-R mRNA regulation (6, 11). These observations on regulation of FGF-R mRNA levels in SC-3 cells seem to be compatible with the studies of FGF-dependent mitogenic signal transduction pathway in mouse 3T3 cells which is similar to but distinct from protein kinase C-dependent pathway (12).

The present study also demonstrated T-induced up-regulation of FGF-R mRNA levels in SC-3 cells. These results confirm our previous proposal that the growth of SC-3 cells is mediated through an androgen-induced FGF-like growth factor (8). Additionally, the evidence presented here suggests that up-regulation of FGF-R mRNA is initiated by binding of FGF-R to its ligand in a relatively stringent way.

ACKNOWLEDGMENTS

We gratefully acknowledge the gift of a chick FGF-R cDNA probe from Dr. L.T. Williams. This work was supported by grants from the Ministry of Education, Tokyo, Japan, Cancer Research Promotion Fund, and Hirai Cancer Research Fund.

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