NUCLEAR ACCUMULATION OF EXOGENOUS BASIC FIBROBLAST GROWTH FACTOR IN ENDOTHELIAL, FIBROBLAST, AND MYOBLAST CELL LINES RESULTS IN DIVERSE BIOLOGICAL RESPONSES

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SUMMARY

During studies comparing 125I-bFGF internalization between endothelial cells and other cell types, we found, unexpectedly, internalization and nuclear translocation of exogenously added 125I-bFGF in two cell lines: Chinese hamster ovary cells (CHO) and rat L6 myoblasts. These cell lines were previously reported to be devoid of FGF receptors. Furthermore, CHO cells showed a weak mitogenic response to added bFGF, while L6 cells were mitogenically unresponsive. By comparison, coronary venular endothelial cells (CVEC), BALB/c 3T3 fibroblasts, and BHK-21 cells, demonstrated internalization and nuclear translocation of added 125I-bFGF, and mitogenic responsiveness to the growth factor. Insulin alone stimulated DNA synthesis in all cell types, yet augmented bFGF-dependent DNA synthesis only in CVEC, 3T3, and BHK. All five cell types expressed FGF receptors as assessed by covalent crosslinking with 125I-bFGF and immunoblotting with anti-FGF receptor antibodies. Differing rates of cytoplasmic and nuclear accumulation of 125I-bFGF and partial inhibition of internalization by pretreatment of CVEC with chlorate support a recent model that bFGF can internalize by two mechanisms. Insulin did not significantly affect 125I-bFGF internalization or metabolism in any cell type. bFGF treatment resulted in weak inhibition of RNA synthesis in L6 cells. bFGF appears firmly bound to the nuclear matrix as little nuclear-bound 125I-bFGF in CVEC is released by DNAse I or RNAse A digestion, while washes with 0.5 M NaCl result in partial release. Nuclear bFGF may thus be involved in regulation of nuclear events (e.g., gene transcription and/or DNA replication).

Key words: insulin; DNA synthesis; bFGF internalization; nuclear targeting of bFGF; FGF receptor; nuclear matrix.

INTRODUCTION

In recent years, it has been shown that exogenously added basic fibroblast growth factor (bFGF) is internalized and translocated to nuclei of endothelial cells (3,5,14) and neurons and astrocytes (46). In addition, endogenous acidic and basic FGF have also been detected in the nuclei of various cell types (9,41) and cells transfected with the cDNA encoding fibroblast growth factor (6,10,17,33,36,44). We and others have proposed that nuclear binding of FGF may play a role in cell signaling and/or proliferation of target cells (1,3,14,16,24).

Because nuclear binding of exogenous bFGF has only been shown in endothelial and neural cells, we investigated internalization and nuclear translocation of 125I-bFGF in other cell types to determine if it was correlated with mitogenic responsiveness. We tested two cell lines, BALB/c 3T3 fibroblasts and baby hamster kidney BHK-21 fibroblasts, previously shown to contain abundant FGF receptors (FGFR) and/or respond mitogenically to added bFGF (22,28,29); and two cell lines, Chinese hamster ovary (CHO) cells and rat L6 myoblasts, that had been previously reported not to express FGFR (20,25,26,30). These four cell lines were compared to coronary venular endothelial cells.

In previous studies on initiation of DNA synthesis in quiescent BALB/c 3T3 cells mediated by growth factors, it was found that cells respond optimally (i.e., maximum DNA synthesis) when exposed to a combination of competence factors (e.g., platelet-derived growth factor or FGF) and progression factors (e.g., insulin, insulin-like growth factor I, or epidermal growth factor) in accordance with the “competence and progression” theory of cell cycle progression (31,43,48). We wondered whether insulin might augment bFGF-induced DNA synthesis in the five cell lines to be tested and whether insulin might affect or increase nuclear translocation of internalized bFGF as a part of its mitogenic effect. Accordingly, we also compared the ability of bFGF and/or insulin to stimulate DNA synthesis in all five cell lines in the same serum-free medium and tested the effect of insulin on 125I-bFGF binding, internalization, and nuclear accumulation. Finally, we analyzed the nature of 125I-bFGF bound to endothelial cell nuclei.

In this report, we show that bFGF and insulin are mitogenic for CVEC, BHK-21, and BALB/c 3T3 cells, while only insulin, but not bFGF, is mitogenic for L6. bFGF is weakly mitogenic for CHO cells, while insulin is strongly mitogenic. Surprisingly, all five cell
FIG. 1. Effect of bFGF and insulin on DNA synthesis in different cell lines. Each cell line was seeded in 48-well plates, serum-starved 72 h in serum-free medium, and then incubated with insulin (5 μg/ml) and/or increasing doses of bFGF (0–50 ng/ml) for 17 h.