Role of \textit{bcl-2} in Growth Factor Triggered Signal Transduction\(^1\)

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Abstract

The role of \textit{bcl-2} in the signal transduction pathway was assessed by studying the inositol phospholipid metabolism in fibroblasts transfected by this oncogene. The rate of accumulation of water soluble inositol phosphates in response to several growth factors was much higher in \textit{bcl-2} transfected NIH3T3 clones than in untransfected control. Moreover, \textit{bcl-2} transfected clones express elevated levels of phosphatidic acid, a phospholipid produced during receptor stimulated breakdown of phosphoinositides. Our findings suggest that the expression of \textit{bcl-2} in NIH3T3 fibroblasts leads to the coupling of growth factor receptors to stimulate inositol phosphate production, henceforth establishing its role in the growth factor receptor mediated signal transduction pathway.

Introduction

The \textit{bcl-2} gene is commonly rearranged and overexpressed in human B-cell lymphomas (1–5). Very little information other than GTP binding activity (6) has been available on the biochemical or functional nature of the proteins encoded by the \textit{bcl-2} gene, whose chromosomal translocation is associated with approximately 85% of follicular lymphomas and at least 10% of B-cell chronic lymphocytic leukemias (1–5). A recent study, however, did not confirm the GTP binding activity of the \textit{bcl-2} product (7). The possible involvement of \textit{bcl-2} in lymphocyte proliferation has been demonstrated recently (8–11). Cell fractionation experiments indicate that \textit{bcl-2a} gene product (p26) is localized on the inner surface of the cell membrane (6, 12). Taken together these studies suggest the potential role of \textit{bcl-2} in signal transduction pathway. Since the inositol phospholipid metabolism is one of the major pathways of signal transduction in cells (13), the role of \textit{bcl-2} in signal transduction was assessed by studying the inositol phospholipid metabolism in fibroblasts transfected by this oncogene.

Materials and Methods

Cell Lines. 596-2 clones are \textit{bcl-2a} DNA transfected NIH3T3 fibroblasts as described (14) whereas 596-1 cells are untransfected control. Both 596-7 (14) and C74-81 are activated ras oncogene transfected NIH3T3 clones.

Measurement of Water Soluble Inositol Phosphates. An identical number of cells grown in 35-mm dishes were starved with inositol free DMEM\(^2\) containing 2.5% dialyzed serum for 3–6 h. Following preincubation with inositol free DMEM, cells were labeled with \textit{myo}-\textit{[3H]}inositol (17.4 Ci/mmol; Amersham) for 16–20 h. \textit{[3H]}inositol labeled cells were harvested, washed 3 times with phosphate buffered saline and resuspended in the same media. An equivalent number of cells were preincubated with 10 mM LiCl before activation with several polypeptide growth factors such as bombesin, bradykinin (Calbiochem), epidermal growth factor, or platelet derived growth factor in a 37°C water bath for 10–30 min. The incubations were terminated by the addition of chloroform:methanol (1:2). Aqueous and organic phases were separated by addition of chloroform and water. The inositol phosphates present in upper aqueous phase were determined by batch chromatography on Dowex 1-X8 formate resins (Bio-Rad). The water soluble \textit{[3H]}inositol phosphates were determined by counting in Beckman Redi solvent scintillation cocktail by liquid scintillation spectroscopy.

Results and Discussion

NIH3T3 fibroblasts were transfected with \textit{bcl-2a} DNA by the calcium phosphate precipitation method as described previously (14). Unlike many other oncogenes that encode membrane-associated proteins, \textit{bcl-2} transfection does not induce focus formation transforming of 3T3 cells \textit{in vitro}, but cells recovered from \textit{bcl-2} mediated tumors \textit{in vivo} exhibit a transformed morphology when reestablished in culture (14). 3T3 fibroblast cell lines are known to exhibit receptors for a variety of growth factors including those for bombesin, platelet derived growth factor, and bradykinin. These growth factors have been found to trigger inositol phospholipid breakdown and generation of second messenger molecules responsible for elevating intracellular free [Ca\textsuperscript{2+}] and activating protein kinase C (15). We have used \textit{bcl-2} transfected (596-2) and untransfected control (596-1) cells to study the cooperation between a wide variety of growth factors and \textit{bcl-2a} (p26) in stimulating inositol phospholipid breakdown.

Lipids from each variety of cells were labeled with \textit{myo}-\textit{[2-
in response to stimulation by growth factors, the accumulation of water soluble inositol phosphates is much higher in fibroblasts expressing \textit{bcl-2} than in untransfected control. In the same experiments we used \textit{ras} transformed fibroblasts 596-7 (14) and C74-81 as positive control (16). It is true, however, that in comparison to \textit{ras} transfected cell lines, \textit{bcl-2} transfection caused lower levels of inositol metabolite production in response to the stimuli. The increase in inositol phospholipid breakdown was apparent only during receptor activation by growth factors and was not accompanied by any increase in basal rates of turnover (data not shown). Moreover the serum dose-response curve for stimulation of inositol phosphates production in both \textit{bcl-2} transfected and untransfected cells revealed a dramatic difference in magnitude for the two cell populations (Fig. 1).

Subsequent measurement of incorporation of $^{32}$P into inositol phospholipids revealed that in \textit{bcl-2} transfected NIH3T3 cells the rate of incorporation into phosphatidic acid was signifcantly higher than in untransfected controls (Fig. 2). Inositol phospholipid metabolism was studied by measuring the incorporation of $^{32}$P into inositol phospholipids. The levels of incorporation in other phospholipids such as phospatidylinositol and phosphatidylcholine were similar in both \textit{bcl-2} transfected and untransfected cells. Although levels of phosphatidylinositol for 16 h. Following incubation the cells were washed for removal of free radioactivity and culture medium. Thereafter, the cells were removed from the dish and were stimulated with appropriate polypeptide and growth factors. In this study saturating concentrations of growth factors were used. Incubations were carried out for 30 min at 37°C in the presence of 10 mM LiCl. This methodology permitted us to measure the accumulation of inositol phosphates. This method has been shown to be most reliable and easily applied measure of receptor stimulated inositol lipid hydrolysis. As shown in Fig. 1 and Table 1,

Table 1  

\begin{table} 
\centering 
\begin{tabular}{|c|c|c|c|} 
\hline 
Ligand & 596-1$^*$ cells & 596-2 cells & C74-81 cells \\
\hline 
Serum (10%) & 2.8 ± 0.4 & 6.4 ± 0.5 & 8.2 ± 0.3 \\
PDGF (2 μM) & 1.4 ± 0.3 & 2.4 ± 0.2 & 3.2 ± 0.1 \\
EGF (100 ng/ml) & 0.9 ± 0.2 & 2.2 ± 0.1 & 0.8 ± 0.3 \\
Bombesin (100 ng/ml) & 1.1 ± 0.2 & 1.3 ± 0.6 & 2.0 ± 0.4 \\
Bradykinin (100 ng/ml) & 2.0 ± 0.1 & 5.0 ± 0.8 & 4.2 ± 0.3 \\
EGF + bombesin & 1.8 ± 0.4 & 3.2 ± 0.2 & 2.7 ± 0.3 \\
\hline 
\end{tabular} 
\caption{Effect of several growth factors on stimulation of phosphatidylinositol metabolism in \textit{bcl-2} and \textit{ras} transfected NIH3T3 cells} 
\end{table}

* 596-1, \textit{bcl-2} untransfected NIH3T3 cells; 596-2, \textit{bcl-2} transfected NIH3T3; C74-81, NIH3T3 cells transfected with activated Ha-ras from the T24 human bladder carcinoma. PDGF, platelet derived growth factor; EGF, epidermal growth factor.

in liquid scintillation spectroscopy. Values represent means of triplicate determinations ± SEM (bars).
dyinositol 4,5-biphosphate were decreased to a significant amount in the case of ras transformed fibroblasts (C74-81). bcl-2 transfection could decrease the phosphatidylinositol 4,5-biphosphate level by much lesser extent (Fig. 2).

In summary, the data presented here clearly indicate that bcl-2 transfection in fibroblasts causes enhanced rate of inositol phospholipid turnover in the form of water soluble inositol phosphates. Furthermore, the increased rate of one phospholipid production is reflected through a significantly higher rate of incorporation into PA extracted from bcl-2 transfected and untransfected clones. In this respect, the enhanced rate of phospholipid turnover in bcl-2 transfected fibroblasts is interesting. Although the role of this intriguing phospholipid as a growth factor is well known (17–20), the mechanism by which PA stimulates phosphoinositide breakdown is still obscure. One possibility is by directly activating phospholipase C or by some indirect effect, e.g., the perturbation in the structure of lipid bilayer, thereby making phosphatidyl inositol 4,5-biphosphate substrate susceptible to phospholipase attack. The second possibility seems more likely. The ability of PA to form non-bilayer domains has been documented (21–23) and phospholipase action is promoted when the bilayer is perturbed (24, 25). If that be the case, then newly synthesized PA due to overexpression of bcl-2α in fibroblasts can exert function in a positive feedback loop to amplify the cascade of inositol lipid interactions following receptor stimulation. Interestingly recent studies (9, 10) indicate that overexpression of bcl-2 gene product results in growth advantages by proliferating in low serum. One might speculate that enhanced rate of inositol metabolites turnover could possibly result in cell growth advantage.

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References

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