Mini Review

Influence of basic fibroblast growth factor in the solution and adsorbed form on the proliferation and differentiation of cells

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There are some action modes to activate the receptor of growth factors and cytokines, such as juxtacrine, autocrine, and paracrine fashions. Some of growth factors transduce the signal to the target cell while anchored (immobilized or adsorbed) to a biological substance. For example, binding of fibroblast growth factor (FGF) to heparin is required for the signal transduction. Biological activity of growth factors, such as epidermal growth factor (EGF), nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and insulin, in the immobilized or absorbed form have been compared with that of factors in the water-soluble form. The growth factors immobilized or adsorbed maintain the activities to transduce the signals for activation of cell functions. The amount of growth factors immobilized to affect the proliferation of cells is smaller than that of water-soluble factors. There is difference in the time profile of the functional activation between the two forms of growth factor. This paper briefly reviews the influence of growth factors on the proliferation of cells while our data about the effect of basic FGF (bFGF) in different modes on the proliferation and differentiation of bone marrow derived stem cells.

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Introduction

Biological significance of growth factors has been increasingly magnified in terms of the medical researches and clinical applications. Many researches have clarified the receptor and signal transduction pathway of growth factors. Among of them, fibroblast growth factors (FGF) family consists of 18 types of FGF and binds 7 types of FGF receptors (FGFR). FGF-induced receptor dimerization is required for the signal transduction[1]. Heparin is required for high affinity binding of basic FGF (bFGF) to the FGFR1 of cells that do not have an ability to synthesize cell-surface heparin sulfate[2,3]. The formation of heparin-induced acidic FGF (aFGF) dimmer is the key step of FGFR2 dimerization[4]. Therefore, the distance between FGF molecules and their anchoring to heparin are important to bind the FGFR and consequently activate the signal pathway (**Fig.1a**). Epidermal growth factor (EGF) and heparin-binding EGF-like growth fac-

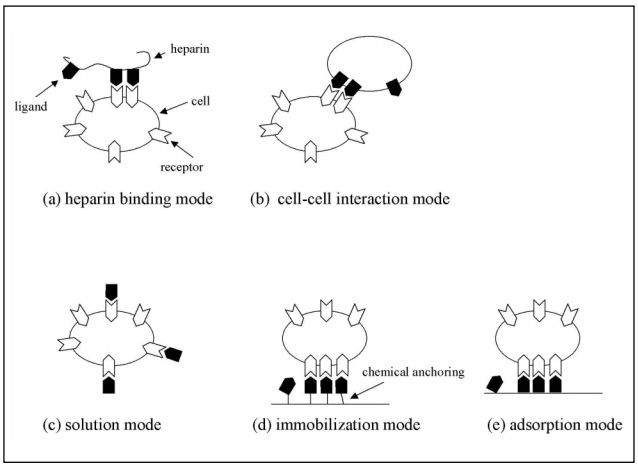

Fig.1 Modes of interaction between growth factor ligands and the receptors

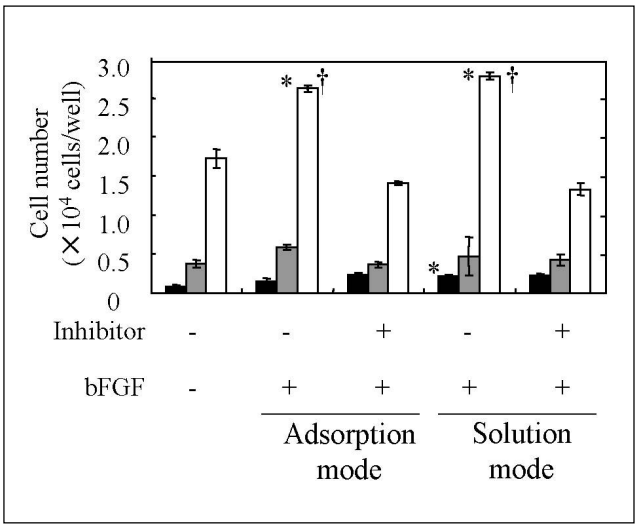

Fig.2 Proliferation profiles of BHK 21 cells cultured in the presence of bFGF adsorbed or soluble mode with or without the inhibitor for 3 (■), 24(■), and 48(□) hrs. BHK 21 cells were incubated with 30 μM of SU5402 for 5 min and seeded on cell culture plate (0.3 cm²) with bFGF in the adsorption (25 ng/cm²) and solution mode (100 ng/ml 75 μl).

***$p<0.05$: significant against the number of cells cultured without bFGF. †$p < 0.05$: significant against the number of cells cultures with bFGF after incubated inhibitor.**

tor (HB-EGF) bind to the epidermal growth factor receptor (EGFR) to activate cellular functions[5,6]. It is reported that HB-EGF acts in the juxtacrine and paracrine fashions. HB-EGF of the former fashion is especially called proHB-EGF. ProHB-EGF is tethered to the plasma membrane of cells and can transmit the signal to the neighboring cell in contrast to the soluble mode (**Fig.1b**). The action of growth factors anchored to a biological substance or cell surface must be distinct from that of growth factors in the soluble form (**Fig.1c**).

Immobilization or adsorption and solution modes of growth factors

Growth factors are immobilized (**Fig.1d**) or adsorbed (**Fig.1e**) onto a solid substrate and the biological function has been compared with that of soluble growth factors (**Fig.1c**). Growth factor and cytokine immobilized or adsorbed on a solid substrate maintain their activity, and for some growth factor and cytokines, the time profile of their activation is different from that of water-soluble form. Chen et al. show that immobilization enabled EGF sufficiently to enhance the biological activity for cell growth of mouse STO fibroblasts and chinese hamster ovary cells (CHO)-K1[7]. Ito et al. have investigated the proliferation of rat pheochromocytoma cell line (PC12) cells and the activation of mitogen-activated protein kinase (MAPK) upon culturing in the presence of EGF in the immobilized and solution forms. Immobilized EGF activated the extracelluar signal-regulated kinase (ERK) and p38 MAPK of cells for longer time periods than soluble EGF[8]. The MAPK activation induced by the immobilized EGF was observed in A431 cells and the activation lasted for a longer time period than that of soluble EGF[9]. Naka et al. have repotted about the neurite outgrowth of PC12 cells cultured on magnetic beads with or without adsorption of nerve growth factor (NGF) or brain-derived neurotrophic factor (BDNF)[10]. The NGF or BDNF-adsorbed beads increased the neurite outgrowth of cells to the same extent as the original beeds together with soluble NGF or BDNF. When the proliferation of CHO-T cells and activation of the insulin receptor, insulin receptor substrate 1, and phoshatidylinositol (PI)-3-kinase were investigated[11,12], both the insulin receptor phosphorylation and PI-3-kinase activity continued to increase over 12 hr for the insulin immobilized mode, in contrast to the soluble-mode insulin. The increased amount of total DNA was observed for insulin immobilized mode in contrast with the soluble insulin one.

There are some methods for artificial anchoring of growth factors. The anchoring methods were independent of the activity of growth factors and the growth factors induce the cell proliferation and differentiation. Irrespective of the immobilized mode, insulin at concentrations higher than 100 ng/cm² was effective in enhancing the cell proliferation[11-13]. The immobilized and adsorbed of bone morphogenic protein-2 (BMP-2) increased alkaline phosphatese (ALP) activity of ST2 cells[14].

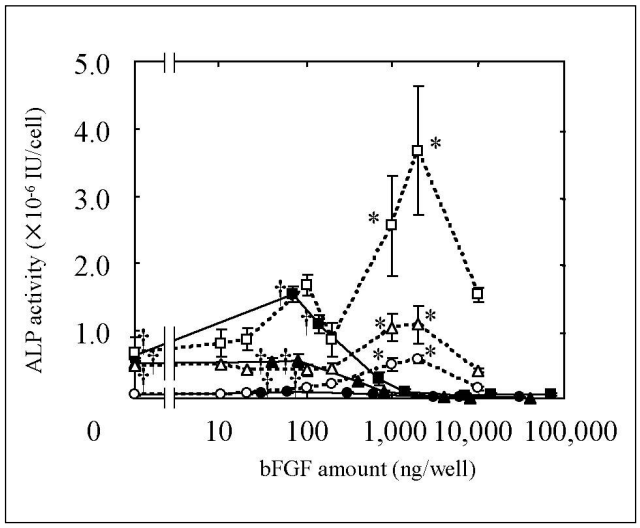

Fig.3 ALP activity of MSC cultured in osteogenic differentiation medium containing different amounts of bFGF for 10 (○, ●), 14(△, ▲), and 21(□, ■) days. bFGF was adsorbed onto the culture plates (2 cm², open symbols) or added to the medium (solid symbols).

*** $p < 0.05$: significant against the ALP activity of MSC cultured in medium containing bFGF in adsorption form at 0, 10, 20 and 100 ng/well. † $p < 0.05$: significant against the ALP activity of MSC cultured in medium containing bFGF solution form at 1,000, 2,000, and 100,000 ng/well.**

Adsorption and solution modes of bFGF

It has been well recognized that bFGF affects the proliferation and differentiation of various cells. There are some research reports on the addition effect of bFGF on the cell proliferation although little has been compared in the cell behavior between the addition modes. Baby hamster kidney (BHK) 21 cells have a FGFR and the proliferation is promoted by bFGF[15]. **Fig.2** shows the proliferation profile of BHK21 cells cultured with bFGF in the adsorption or solution mode together with the addition effect of a FGFR1 inhibitor, SU5402[16]. Irrespective of the bFGF addition mode, bFGF presence increased the number of BHK 21 cells proliferated to a significant great extent compared with bFGF-free culture. A similar inhibition effect of SU5402 on the cell proliferation was observed for both the modes of bFGF. Taken together, it was found that bFGF adsorbed maintained the activity similar to that of free bFGF. BHK 21 cells have 60,000 sites of FGFR per cell to bind bFGF[15]. If we assume that cell spread-area is 600-1000 μm^2 and FGFR assemble in the side immobilized bFGF, BHK 21 cells would have FGFR at the density of 6×10^9-1×10^{10} receptor/cm². Three-dimensional structure of bFGF was measured by x-ray. The cell dimension of bFGF crystals was about 3 nm[17]. If bFGF is adsorbed in a close packed state, the density of bFGF would be 1×10^{13} ligand/cm². bFGF adsorbed under our condition was 2.5 ng/cm², therefore 8×10^{11} ligand/cm² (18kDa)[18]. Therefore, when bFGF was adsorbed in a monolayer fashion, it is the number of ligand enough to bind the receptors of BHK 21 cells.

Fig.3 shows the level of ALP activity of rat bone marrow stromal cells (MSC) cultured in osteogenic differentiation medium containing bFGF in the adsorption or solution mode. The adsorption mode enabled MSC to significantly increase the ALP activity level compared with the solution mode. The level tended to increase with the amount of bFGF adsorbed, and attained to a maximum at the 10,000 ng/well over the time period studied. On the other hand, the opposite concentration dependence was observed for bFGF in the solution mode. The ALP activity decreased with the bFGF amount up to 1,000 ng/well, and thereafter became almost zero. Some researches have investigated the effect of bFGF addition into an osteogenic differentiation medium on the osteogenic differentiation of MSC. Locklin et al. revealed that the presence of bFGF enhanced the proliferation of MSC, but suppressed the osteogenic differentiation[19]. On the other hand, Frank et al. showed that bFGF induced BMP-2 expression[20]. These findings indicate that osteogenic differentiation of MSC was influenced by the concentration of bFGF and the addition mode.

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