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ORIGINAL ARTICLE

Diversity of bone matrix adhesion proteins modulates osteoblast attachment and organization of actin cytoskeleton

Les différents types de molécules d'adhésion modulent l'ancrage des ostéoblastes et l'organisation des fibres d'actine du cytosquelette

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Matrix protein;
Osteoblast;
Cytoskeleton;
Scanning electron microscopy;
Immunogold labeling;
Fractal analysis;
Vitronectin;
Integrin;
Fibronectin

Summary Interaction of cells with extracellular matrix is an essential event for differentiation, proliferation and activity of osteoblasts. In bone, binding of osteoblasts to bone matrix is required to determine specific activities of the cells and to synthesize matrix bone proteins. Integrins are the major cell receptors involved in the cell linkage to matrix proteins such as fibronectin, type I collagen and vitronectin, via the RGD-sequences. In this study, cultures of osteoblast-like cells (Saos-2) were done on coated glass coverslips in various culture conditions: DMEM alone or DMEM supplemented with poly-L-lysine (PL), fetal calf serum (FCS), fibronectin (FN), vitronectin (VN) and type I collagen (Col-I). The aim of the study was to determine the specific effect of these bone matrix proteins on cell adherence and morphology and on the cytoskeleton status. Morphological characteristics of cultured cells were studied using scanning electron microscopy and image analysis. The heterogeneity of cytoskeleton was studied using fractal analysis (skyscrapers and blanket algorithms) after specific preparation of cells to expose the cytoskeleton. FAK and MAPK signaling pathways were studied by western blotting in these various culture conditions. Results demonstrated that cell adhesion was reduced with PL and VN after 240 min. After 60 min of adhesion, cytoskeleton organization was enhanced with FN, VN and Col-I. No difference in FAK phosphorylation was observed but MAPK phosphorylation was modulated by specific adhesion on extracellular proteins. These results indicate that

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culture conditions modulate cell adhesion, cytoskeleton organization and intracellular protein pathways according to extracellular proteins present for adhesion.

MOTS CLÉS
Protéines matricielles ;
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Immunomarquage à l'or ;
Analyse fractale ;
Vitronectine ;
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Fibronectine

Résumé L'interaction des cellules avec la matrice extracellulaire est un événement essentiel pour la différenciation, la prolifération et l'activité des ostéoblastes. Dans l'os, l'adhérence des ostéoblastes à la matrice osseuse est nécessaire pour déterminer les activités spécifiques de ces cellules et pour qu'elles synthétisent les protéines matricielles. Les intégrines sont les principaux récepteurs cellulaires impliqués dans la liaison des cellules aux protéines matricielles telles que la fibronectine, le collagène de type I et la vitronectine (via la séquence RGD). Dans cette étude, des cultures de cellules de type ostéoblastique (Saos-2) ont été réalisées sur des lamelles de verre recouvertes de différents supports de culture : DMEM seul ou DMEM supplémenté avec de la poly-L-lysine (PL), sérum de veau fœtal (FCS), fibronectine (FN), vitronectine (VN) et collagène de type I (Col-I). Le but de l'étude était de déterminer l'effet spécifique de ces protéines matricielles sur l'adhérence des cellules, leur morphologie et sur leur cytosquelette. Les caractéristiques morphologiques des cellules en culture ont été étudiées en microscopie électronique à balayage et analyse d'images. L'hétérogénéité du cytosquelette a été étudiée par analyse fractale (algorithmes des gratte-ciel et des couvertures) après une préparation spécifique des cellules pour exposer le cytosquelette. Les voies de signalisation FAK et MAPK ont été étudiées par western blot dans ces différentes conditions de culture. Les résultats ont montré que l'adhésion cellulaire a été réduite avec PL et VN après 240 minutes. Après 60 minutes, l'organisation du cytosquelette a été optimisée sur les lamelles recouvertes de FN, VN et Col-I. Aucune différence dans la phosphorylation de FAK n'a été observée mais la phosphorylation MAPK était modulée par l'adhérence spécifique aux protéines extracellulaires. Ces résultats indiquent que les conditions de culture modulent l'adhésion cellulaire, l'organisation du cytosquelette et les voies de signalisation intracellulaire en fonction des protéines extracellulaires présentes qui permettent l'adhérence des cellules ostéoblastiques.

Introduction

Osteoblasts are the cells responsible for bone formation. They synthesize numerous proteins of the extracellular bone matrix and are involved in the process of mineralization. Osteoblasts are generated from bone marrow osteoprogenitors and several hormones and growth factors contribute to their differentiation and activity. However, interaction of cells with the extracellular matrix is an essential event for differentiation, proliferation and activity of osteoblasts [1]. Osteoblasts are linked to the bone matrix via cell surface receptors (mainly integrin) that bind the specific arg-gly-asp amino-acid sequence (RGD) shared by some glycoproteins of the matrix (thrombospondin, fibronectin, vitronectin, type I collagen, fibrilline and osteopontin, bone sialoprotein [belonging to the SIBLING family: small integrin-binding ligand, N-linked glycoproteins]) [2]. Integrins are $\alpha\beta$ heterodimers with an extracellular domain that binds the RGD sequence, a transmembranous part and a short intracellular sequence linked to cytoskeleton proteins. At least 16 α subunits and 8 β subunits have been identified leading to more than 20 different types of integrins. Each type of integrin is able to bind a specific RGD-containing protein but several integrins may also bind the same protein [3]. The major cell vitronectin receptor is $\alpha_V\beta_3$ that can also bind fibronectin (FN); conversely, vitronectin (VN) may also link other integrins. Numerous integrins mediate cell attachment to FN, among them $\alpha_3\beta_1$, $\alpha_4\beta_1$ and $\alpha_V\beta_3$ [4]. All these integrins are expressed at the osteoblast surface [5]. However, in all human osteosarcoma cells some of them may be absent. For example, Saos-2 cells express $\alpha_1\beta_1$ but not $\alpha_2\beta_1$ [6] contrary to MG-63 where only $\alpha_2\beta_1$ (and not $\alpha_1\beta_1$) is present as type I collagen receptor [7].

When bound to their ligands, integrins transmit signals by recruiting cytoskeleton and signaling proteins to sites known as focal adhesions. Formation of focal adhesion provides the physical contact sites of attachment between cells and extracellular matrix. Clustering of integrins in focal adhesion site induces the recruitment and the phosphorylation of tensin and focal adhesion kinase (FAK) that subsequently induces the recruitment of talin, vinculin and α-actinin [8,9]. Talin, vinculin and α-actinin link the F-actin fibers to the plasma membrane and interact with several proteins such as vaso-dilatator phosphoprotein (VASP) and Arp2/3 proteins which are implicated in the regulation of actin polymerization [10,11]. Rearrangement of F-actin bundles induced by their liaison to the integrin β subunit can induce changes in the cell shape [12,13]. This can produce finger-like or sheet-like protrusions (respectively filopodia and lamellipodia) [14].

Synthesis and distribution of cytoskeleton proteins have been involved as a cause or a consequence, in osteoblast morphology, growth, migration, attachment, signaling and functions [15]. The growth of bone cells is associated with changes in organization and synthesis of cytoskeleton proteins and migration implicates dynamic reorganization of the cytoskeleton. Rearrangement and polymerization of actin filaments are a consequence of the linkage between cell surface receptor and bone matrix adhesion protein. As a consequence of the involvement of osteoblast attachment via the integrin-cytoskeleton system, signal transduction events are activated allowing control of gene expression

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