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Review

Cell adhesion molecules in human osteoblasts: structure and function

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Summary. Osteoblasts and bone lining cells form a near continuous layer covering the bone surface and interactions between these cells and the organic matrix of bone are important determinants of osteoblast proliferation and differentiation. In addition, cells of the osteoblast-lineage form functional communications with each other, with the extra-cellular matrix and with osteocytes through cytoplasmic processes extending through canaliculi in the bone. Together, these cells form a network of putative importance in the regulation of skeletal homeostasis. Cell-cell and cell-matrix interactions are mediated by members of several families of cell adhesion molecules, and knowledge of their interactions will be of fundamental importance in understanding the role of osteoblast in skeletal turnover . Here, the expression pattern of members of the major families of cell adhesion molecules by cells of the osteoblast lineage is reviewed. Special emphasis has been placed on human tissues. In addition, the possibility that cells at progressive stages of the osteoblast lineage have different profiles of cell adhesion molecule expression is explored, and the putative significance of cell-matrix interactions in human skeletal disease briefly discussed.

Key words: Adhesion, Cadherin, Differentiation, Integrin, Osteoblast

Adhesion and osteoblast function; an overview

Bone remodeling involves the co-ordinated response of osteoblasts, osteocytes and osteoclasts. Osteoblasts and bone lining cells form a continuous layer covering the periosteal, endosteal and trabecular bone whilst osteocytes are found in lacunae set within the bone matrix and are joined both to their neighbours and cells lining the bone surfaces by cytoplasmic processes which pass through fine channels or canaliculi. Osteoclasts are recruited, when needed, to sites of bone resorption. Together, this interconnecting network of osteoblasts, bone lining cells and osteocytes provide a possible mechanism for the detection of physical or mechanical changes and the co-ordination of resorptive and osteosynthetic activity leading to remodeling. Cell-cell and cell-matrix communication is central to this process.

Pathological situations where the balance between resorption and remodelling becomes disturbed further illustrate the importance of cell-matrix interactions in skeletal homeostasis. In the adult, bone formation *de novo* is pathologic or metaplastic. Under normal circumstances osteosynthesis takes place during remodelling of pre-existing bony surfaces. If these are lost, as in post menopausal osteoporosis, bone formation does not occur, the trabecular structure breaks down and can become replaced by fatty tissue (Burkhardt et al., 1987). Several studies have demonstrated the presence of pluripotential stromal-vascular precursor cells capable of giving rise to osteoblast-like cells and adipocytes within the marrow (Friedenstein, 1976; Owen, 1985; Bennett et al., 1991). It is probable that interactions between these cells and components of the trabecular bone matrix are determining factors in bone cell recruitment.

Cell-matrix interactions associated with osteoclastic bone resorption have been researched extensively (Väänänen and Horton, 1995; Horton and Rodan, 1996). Much less is known about cell-cell and cell-matrix interactions in osteoblasts and related populations, although there has been considerable progress since the first published analysis (Horton and Davies, 1989). Here, we review the status of knowledge of the role of the major families of cell adhesion molecules in cells of the osteoblast lineage both in health and disease.

Integrins

Structure and function

Integrins (Hynes, 1987, 1992; Ruoslahti and Pierschbacher, 1987) are N-glycosylated glycoproteins with a heterodimeric structure composed of covalently

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linked α and ß chains. 16 different mammalian α and 8 ß subunits have been identified, combinations of which form 22 distinct heterodimers. Most integrins, including all those expressed in osteoblasts, have a transmembrane structure with a large extracellular domain, a single hydrophobic transmembrane region and a short cytoplasmic domain. The extracellular domain has a role in adhesion to extra-cellular matrix proteins which involves binding highly specific peptide recognition sequences such as the Arg-Gly-Asp (RGD) sequence present in fibronectin or osteopontin (Pierschbacher and Ruoslahti, 1984) or the Gly-Glu-Arg (GER) motif in type I collagen (Knight et al., 1998). Ligand binding leads to activation of one or more intracellular signal transduction pathways which, in turn, contribute to the regulation of differentiation, cytoskeletal organisation and other aspects of cell behaviour. In this way cells can respond to signals in the surrounding extra-cellular matrix, so called 'outside-in' signalling. In addition, a cell can determine the way it responds to the surrounding matrix through regulation of integrin synthesis and activity, a process termed 'inside-out' signalling. These pathways have been reviewed extensively in this journal (Cary et al., 1999) and elsewhere (Clark and Brugge, 1995; Schlaepfer and Hunter, 1998; Dedhar et al., 1999; Coppolino and Dedhar, 2000).

There is now a growing body of evidence which points to a complex functional association between integrin-ligand binding and the regulation of cell behavior. Integrin-ligand binding, for example, can stimulate protease synthesis (Huhtala et al., 1995) and connective tissue breakdown. This may lead to exposure of ligand binding sites, altered cell-matrix signaling and modification of cell behavior (Werb, 1997). Evidence is also accumulating for a functional relationship between integrins and growth factors (Miyamoto et al., 1996; Sastry and Horwitz, 1996; Takeuchi et al., 1996; Schneller et al., 1997; Woodard et al., 1998; Giancotti and Ruoslahti, 1999). Specific examples of situations in which growth factor receptor function is related to integrin heterodimers are discussed in later sections. Modulation of cell behavior, therefore, is unlikely to depend on signals from a single integrin. Instead, the product of convergence and summation of signals from several sources, including growth factors and extra-cellular matrix receptors, contribute to the homeostatic regulation of cell function. Although, to date, few studies have been directed specifically to osteoblasts, it would indeed be surprising if this were not true for cells of the osteoblast lineage.

Integrins and bone

Bone cells express a diverse range of integrins. Cells of the two major bone lineages, the osteoblasts and osteoclasts, show distinct but overlapping patterns of integrin heterodimer expression. The αvß3 together with the α2ß1 heterodimers, are associated with osteoclast activity (Nesbitt et al., 1993; Väänänen and Horton, 1995; Helfrich et al., 1996; Horton and Rodan, 1996; Horton, 1997; Helfrich and Horton, 1999). Cells of the osteoblast lineage express a rich array of integrins (Table 1) with some contradiction between different studies as

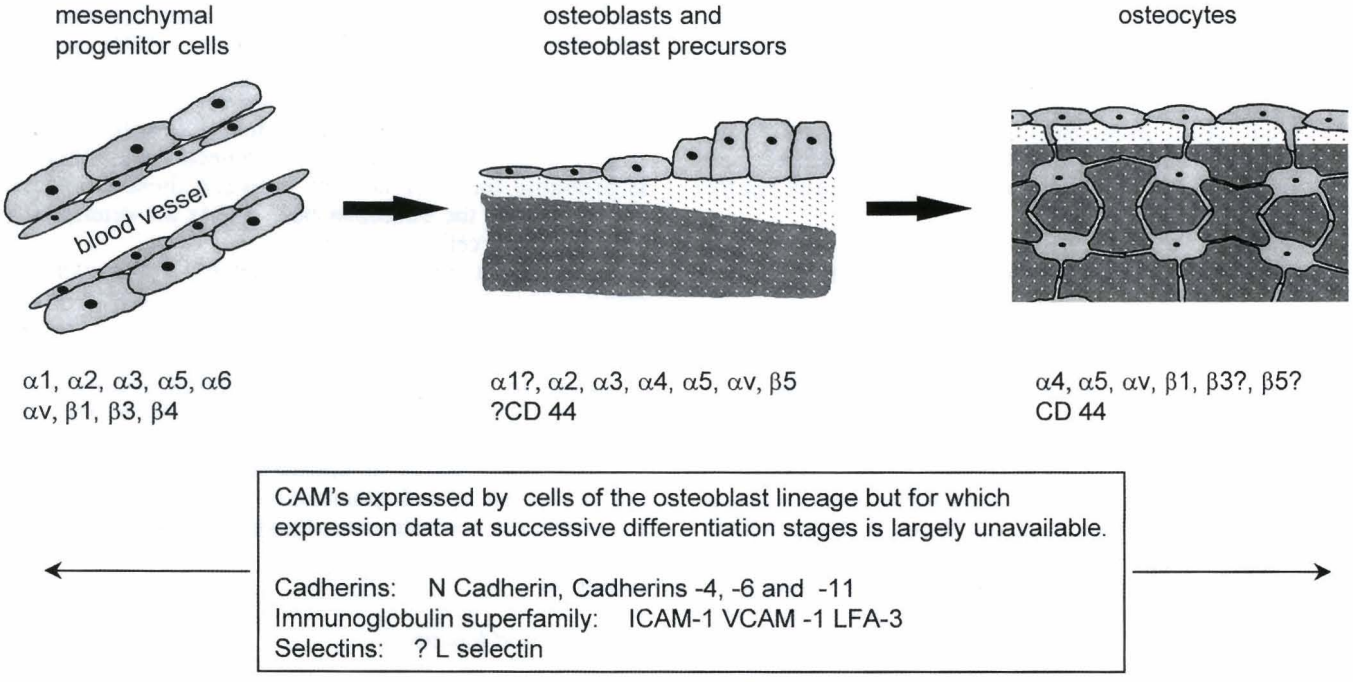

Fig. 1. A diagramatic representation of a possible pattern of cell adhesion molecule (CAM) expression by cells at progressive stages of the osteoblast lineage.

to the specific heterodimers expressed. This may reflect the heterogeneity of osteoblast-like populations and includes the possibility that cells at successive stages of osteoblast differentiation, from foetal or adult bone, or from different anatomical sites, show different pattens of integrin expression. Nevertheless, trends are emerging. Whilst they may express αv integrins, osteoblastic cells differ from osteoclasts in that the ß1 integrins appear to have the major functional role. Several ß1 integrins are expressed (Table 1) and include heterodimers with a high affinity for extra-cellular matrix components found in the matrix adjacent to bone such as collagen types I and III, osteopontin and fibronectin.

Osteoblasts are uniquely involved with the synthesis and maintainence of the bone matrix, and the recognition of specific matrix components by integrin receptors appears to be necessary for osteoblast differentiation and maturation into osteocytes. However, it has proved difficult to study the role of individual integrins *in vivo* using contemporary transgenic strategies as loss of some integrins, particularly the ß1, α3 and α5 chains, leads to pre-natal mortality at a stage of skeletal immaturity (Faessler and Meyer, 1995; Faessler et al., 1996). To overcome this problem, ß1-null embryonic stem cells have been generated and implanted into mice to give ß1-null teratomas or chimeras. Although not specifically directed towards skeletal tissue, these showed alterations in cell adhesive properties and extra-cellular matrix deposition (Brakebush et al., 1997). An alternative approach has involved expression of a dominant negative ß1 integrin subunit which is driven by an osteocalcin promoter (Zimmerman et al., 2000). Osteocalcin is a late stage marker of osteoblastic differentiation so the dominant negative ß1 subunit was only expressed in mature, rather than developing osteoblasts. Transgenic animals showed a decreased bone mass with osteoporotic changes in both the long and membrane bones, thus confirming a role for the ß1 subunit in adult bone turnover. The application of similar strategies to other integrin subunits will be required to determine their role *in vivo*.

Collagen receptors

The α1ß1, α2ß1 and α3ß1 integrins bind collagen and both fluorescent activated cell sorting (FACS) and immunohistochemical studies confirm that they are expressed by osteoblastic cells *in vivo* and *in vitro*. Collagen type 1 is the dominant bone matrix protein and interactions involving these receptors are strong candidates for a role in regulating osteoblast behaviour. Furthermore, functional studies have demonstrated that α2ß1-ligand binding leads to expression of markers of osteoblastic differentiation such as cbfa-1, alkaline phosphatase or osteocalcin (Xiao et al., 1997, 1998). Culture of osteoblast-like cells in the presence of specific inhibitors or α2 function-perturbing antibodies blocks α2ß1 dependent expression of these markers (Takeuchi et al., 1997; Jikko et al., 1999). Others have shown that α2 integrin ligand binding modulates cell motility and contraction of collagen gels *in vitro* (Riikonen et al., 1995: Moffatt et al., 2000).

α3ß1 heterodimers bind collagen, laminin, fibronectin and several other extra-cellular matrix proteins including osteopontin and bone sialoprotein (Isacke and Horton, 2000). Both RGD and non-RGD dependent mechanisms are involved. The α3ß1 integrin is expressed by human bone cells (Table 1) but in osteoblastic cells rather than earlier, less differentiated

Table 1. A summary of results compounded from key immunohistochemical and FACS studies on the expression of integrins in human osteoblasts. Bracketed citations report negative results. * indicates equivocal results.

	IMMUNOHISTOCHEMICAL STUDIES		IN VITRO STUDIES	
	Osteoblasts	Osteocytes	Osteoblast-like cells	Mesenchymal precurso cells
α1 (CD49a)	d (l, k)	(l)	d, b, n, o, i	c
α2 (CD49b)	k, r (l, d)	(l, r)	q, n, o, s, i	c
α3 (CD49c)	(j, l) d	(l)	d, q, n, o, s, i (j)	c
α4 (CD49d)	j, l (d, k)	j, l*	j, o, e (d, i)	(c)
α5 (CD49e)	j, l, k, r (d)	j, l	j, q, o, e, p, i (d)	c
α6 (CD49f)	(l, d, k)	(l)	q, n (d, e, i)	c
αv (CD 51)	j, l (d)	j, l*	j, d, q, e, p	c
ß1 (CD29)	l, d, k	l, a, g	d, q, g, p, i	c
ß2 (CD18)	(d, k)	(l)	(d, e)	(c)
ß3 (CD 61)	(l, d, k)	(l)	j, q, i (d, e)	c
ß4 (CD 104)	(l, k)	(l)	ND	c
ß5	m	j, f*	j, n, o, p, i (f)	ND
αvb1 (CD 51/CD 29)	ND	ND	(o)	ND
αvb3 (CD 51/CD 61)	(l, k)	(l)	n, o*, i	ND
αL (CD 11a)	(d, e)	(d, e)	ND	(c)
αM (CD 11b)	(d, e)	(d, e)	ND	ND

a: Aarden et al. (1996); b: Brighton and Albelda (1992); c: Bruder et al. (1998); d: Clover et al. (1992); e: Clover and Gowen (1994); f: Ganta et al. (1997); g: Gohel et al. (1995); h: Gronowicz and McCarthy (1996); i: Gronthos et al. (1997); j:Grzesik and Robey (1994); k,:Horton and Davies (1989); l: Hughes et al. (1993); m: Hultenby et al. (1993); n: Nissinen et al. (1997); o: Saito et al. (1994); p: Salter et al. (1997); q: Sinha and Tuan (1996); r: Steffensen et al. (1992); s: Riikonen et al. (1995).

populations (Alavi et al., 1998). Function-perturbing antibodies against the α3 integrin significantly inhibit the formation of mineralised nodules in rat calvarial cultures (Moursi et al., 1997).

Integrins and fibronectin

Ten integrins are fibronectin receptors (Isacke and Horton, 2000). Of these, the α3ß1, α4ß1, α5ß1, αvß3 and αvß5 heterodimers are expressed in bone, but there is controversy about expression of the αvß3 receptors by osteoblastic cells as opposed to osteoclasts (Table 1). Most are capable of binding a broad range of extra-cellular matrix proteins using both RGD-dependent and independent mechanisms. However, the α5ß1 receptor is unique as fibronectin is its only known ligand.

In vitro studies using a rodent calvarial cell model have shown the α5ß1 receptor to be expressed by cells of osteoblast lineage and there is evidence that it is important in both the development and maintenance of bone. Binding involves at least 2 sequences located in the central cell binding domain of the fibronectin molecule, in particular an RGD sequence located in the type III repeat 10 ($FNIII^{10}$) of the central cell binding region (Pierschbacher and Ruoslahti, 1984; Aota et al., 1994). Interruption of binding with blocking antibodies leads to inhibition of bone nodule formation by osteoprogenitor cells (Moursi et al., 1996, 1997). In mature cells α5ß1-ligand binding appears to be necessary for cell survival and receptor blocking leads to apoptosis (Globus et al., 1998).

The reported expression pattern of α5 integrin in human tissues remains controversial (Table 1) and in our experience, is weak in tissue sections (Bennett et al., in press). Fibronectin is a normal constituent of human bone but data on its distribution within the bone matrix is sparse, and it has been reported absent from mature lamellar bone (Carter et al., 1991). Supporting evidence from rodent tissues suggest that fibronectin synthesis and expression is restricted to developing or immature bone (Weiss and Reddi, 1980; Cowles et al., 1998). It is, therefore, possible that α5ß1-ligand interaction is a feature of bone formation during development or repair, and may not play a prominent role in the turnover and maintenance of mature lamellar bone.

The α4ß1 receptor binds fibronectin in a non-RGD dependent manner, binding to a site in the CS-1 region of the molecule with secondary binding sites in the CS5 and Hep11 regions (Isacke and Horton, 2000). Immunohistochemical studies using rabbit polyclonal antibodies have noted α4 integrin in both osteoblasts and osteocytes (Grzesik and Robey, 1994; Pistone et al., 1996) but this was not confirmed using murine monoclonal antibodies (Clover et al., 1992; Hughes et al., 1993; Alavi et al., 1998).

Integrins and mesenchymal precursor cells

Although the α1ß1 and α2ß1 integrins are both collagen receptors they recognise types I and IV collagens using different mechanisms (Kapyla et al., 2000). In addition, the α2ß1 receptor shows greater affinity for collagen type I and α1ß1 for collagen type IV (Kern et al., 1993). Type IV collagen is a feature of the endothelial basal lamina and the presence of the α1 integrin has been reported in mesenchymal stem cells with osteogenic potential (Bruder et al., 1998a). In addition, both type IV collagen and laminin are synthesised by pericytes (Schor and Canfield, 1998) which have been suggested as putative osteogenic precursor cells, and expression of these proteins is lost as cells become osteogenic (Owen, 1998). It is feasible, therefore, that the α1ß1 heterodimer has a role in cell-matrix interactions involving mesenchymal precursor cells associated with small blood vessels. Support for the hypothesis that mesenchymal osteogenic cells express a profile of extra-cellular matrix receptors favouring adhesion to components of endothelial basal laminae comes from the demonstration that osteoprogenitors, in contrast to calvarial-derived cells, showed preferential binding to laminin, a component of the endothelial basement membrane (Roche et al., 1999). Furthermore, mesenchymal precursor cells express the α6 integrin (Bruder et al., 1998a) which dimerises with both the ß1 and ß4 chains to give specific receptors for laminin, a major component of the basement membrane matrix.

The αv integrins

The αvß3 heterodimer is an RGD-dependent vitronectin receptor. Binding is not restricted to this protein and may involve others with RGD binding sites including fibronectin, osteopontin or bone sialoprotein. The αvß5 heterodimer is also an RGD-dependent vitronectin receptor (Cheresh et al., 1989; Ramaswamy and Hemler, 1990; Horton, 1997) but rather less is known about its biological role. Their distribution and role in osteoblastic cells remains to be fully elucidated. Several studies report expression of the αv in cells of the osteoblast lineage chain but vary with regard to ß3 and ß5 subunits (Table 1), and staining appears more prominent in osteoblasts than osteocytes (Hughes et al., 1993; Grzesik and Robey, 1994).

Some insight into the putative role of the αv integrins may be gained from other systems. The αvß3 heterodimer mediates attachment at sites of focal cell-matrix interactions in cultured fibroblasts (Wennerberg et al., 1996) and osteoclasts (Väänänen and Horton, 1995) but this has not been fully investigated in osteoblasts. In addition to forming complexes with proteins important in intracellular signalling (Cary et al., 1999), it co-localises with the platelet derived growth factor (PDGF) receptor (Vuori and Ruoslahti, 1994; Rousseau et al., 1997), and vitronectin binding enhances the biological activity of PDGF-ß (Schneller et al., 1997; Woodard et al., 1998). Several studies have demonstrated important interactions between vascular endothelial growth factor (VEGF), its receptors, and αv integrins, during induction of angiogenesis. VEGF

activity has been found to depend on cell-matrix interactions involving the αvß5 (Bloch et al., 1997) and ß1 integrins (Friedlander et al., 1995) and the αvß3 receptor has a role in the activation of VGEF receptor-2 (Soldi et al., 1999). VGEF receptors are expressed in cells of the osteoblast lineage and have a regulatory role in neo-angiogenesis during endochondral ossification (Gerber et al., 1999). In the adult, fracture repair involves both endochondral and intramembranous ossification (Einhorn, 1998), and the αvß3 receptor may have a role in facilitating the response of specific regulatory growth factors. Determination of this role will require further studies on the co-localization of integrin receptors and growth factors, and the role of integrin binding on growth factor receptor activation in cells of the osteoblast lineage in normal bone development and pathologic states.

Non integrin cell adhesion molecules

Cadherins

Osteoblasts and bone lining cells form gap and cell junctions of the adherens type with each other and with the osteocytes (Doty, 1981; Palumbo et al., 1990). The cadherins are amongst the best characterised cell-cell adhesion molecules (Takeichi, 1998), are localised to sites of inter-cellular attachment and may, therefore, contribute to the attachment apparatus. A considerable number have been cloned (Isacke and Horton, 2000) and most tissues express several but not all cadherin types. Structurally, they are transmembrane glycoproteins involved in calcium dependent homotypic cell-cell adhesion. They are composed of an extracellular domain of repeated cadherin motifs which interact with cadherins on adjacent cells, a transmembrane domain and a highly conserved cytoplasmic domain. The latter associates indirectly with the actin cytoskeleton through the catenin family of proteins and is involved in signal transduction (reviewed by Takeichi, 1995).

Cells of the osteoblast lineage express a limited repertoire of cadherins including N-cadherin, cadherin-4 and cadherin-11 (Okazaki et al., 1994; Tanihara et al., 1994; Cheng et al., 1998; Ferrari et al., 2000). Primary human osteoblasts and bone marrow precursor cells express cadherin-11 and N-cadherin *in vitro* (Cheng et al., 1998). However, N-cadherin has been histochemically localised to well-differentiated osteoblasts lining the bone surface, but not osteocytes, in foetal rat calvaria. Blocking N-cadherin binding decreased bone nodule formation *in vitro* (Ferrari et al., 2000). There is also evidence that bone marrow stromal cells together with some human transformed osteoblast-like cell lines express cadherin-6 (Mbalaviele et al., 1998). Cadherin-11, alternatively termed osteoblast (OB) cadherin, has 3 isoforms, an intact form which shares 50% homology with N-cadherin, a variant form resulting from alternate splicing and a secreted form which arises from proteolysis of the intact molecule (Kawaguchi et al., 1999). The variant form shows no homophilic cell-cell adhesion properties, although it may assist in the adhesion of the intact form if both are co-expressed.

The biological significance of cadherin mediated cell-cell interactions in bone remains largely unexplored. However there is evidence that cadherins function synergistically with other cell adhesion molecules to influence cell behaviour. They may, for example, modulate the function of connexins, proteins associated with gap junction function (Jongen et al., 1991; Meyer et al., 1992) and osteoblasts are known to communicate via such mechanisms (Yellowley et al., 2000). In common with other cell systems, variations in cadherin expression have been observed in association with malignancy. Reduced N-cadherin and anomalous cadherin 11 expression have been associated with high grade metastatic osteosarcomas (Kashima et al., 1999), suggesting an important role for these molecules in intercellular adhesion.

CD 44

CD 44 is a multifunctional cell surface glycoprotein that binds to hyaluronic acid, collagen types I and IV and fibronectin. Immunohistochemical studies using several antibodies on both frozen and formaldehyde-fixed, paraffin-embedded decalcified sections of human tissue showed CD 44 expression in osteocytes but not osteoblasts or bone lining cells (Hughes et al., 1994). This is consistent with other mammalian models in which strong expression has been observed in osteocytes with rather weaker staining in cells earlier in the osteoblast lineage (Nakamura et al., 1995; Noonan et al. 1996). The functional significance of CD 44 expression is unknown. However, the restriction of its expression to osteocytes points to an important role in cell-matrix interactions associated with bone homeostasis in the adult.

Immunoglobulin superfamily

Although sparse, there is data relating to expression of members of this family by osteoblasts and related cells. ICAM-1 and -2, VCAM-1 and LFA-3 expression has been reported in human bone cells (Tanaka et al., 1995; Bruder et al., 1998a). Expression of activated leucocyte cell adhesion molecule (ALCAM) has been reported in undifferentiated human mesenchymal cells and may have a functional role in osteoblast differentiation (Bruder et al., 1998a,b).

Selectins

There is, as yet, little information on the expression of members of the selectin family in osteoblasts. However, there is evidence for L-selectin expression by human mesenchymal stem cells (Bruder et al., 1998a) but not E- or P-selectin. These molecules are normally associated with leucocyte trafficking across endothelial

membranes and it has been suggested they have a putative role in the extravasation of osteoblast precursors (Helfrich and Horton, 1999).

Syndecans

The Syndecans are a family of membrane bound heparan sulphate proteoglycans. Expression is normally associated with cartilage, but syndecans -1, -2 and -4 have been identified in human marrow stromal and osteoblast-like cells *in vivo* and *in vitro* (Birch and Skerry, 1999; Schofield et al., 1999). Studies using a rat organ culture model demonstrated co-incident expression of syndecans -2 and -4 with fibroblast growth factor receptors -1, -2 and -3 *in vitro* and a similar spatio-temporal expression *in vivo* (Molteni et al., 1999). This suggests a role for members of the syndecan family in the presentation of growth factors during skeletal development but this remains to be investigated.

Concluding comments

Cells of the osteoblast lineage express a wide array of cell adhesion molecules. As in other systems, these provide a conduit for the transmission of signals from the extracellular environment to the cell. However, there is still no clear consensus as to which cell adhesion molecules are expressed by cells of the osteoblast lineage, or whether cells at progressive stages of differentiation show different patterns of expression. Nevertheless, trends are emerging (Fig. 1). Integrins binding laminin or showing a greater affinity for collagen type IV are features of mesenchymal osteoblastic progenitors whilst osteocytes show reduced integrin expression and increased expression of the CD44 hyaluronan receptor. More expression data is needed and the functional significance of these observations requires further investigation.

Osteoblast-matrix interactions may turn out to be of considerable significance in understanding human skeletal diseases. Several conditions are characterised by abnormalities in the extra-cellular matrix and osteosynthetic activity. In osteogenesis imperfecta there are abnormalities of the bone matrix and osteoblast function (Rauch et al., 2000), whilst in Pagets disease of bone, disturbances of both collagenous and non collagenous matrix proteins have been reported, and that formed is reminiscent of foetal rather than the lamellar bone found in the adult (Robey and Bianco, 1999). Complex genetic factors are determinants of fracture risk (Deng et al., 2000; Nguyen and Eisman, 2000) and it is possible that the effect of these is due to subtle abnormalities of the bone matrix. It would not be surprising, therefore, if, in some of these situations, altered signaling between the matrix and the cell did not influence osteoblast behavior. Elucidation of the role of cell-matrix interactions in the aetiology of skeletal disease will, therefore, remain a research challenge for the foreseeable future.

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