COMMENTARY

Genetic analysis of β1 integrin function: confirmed, new and revised roles for a crucial family of cell adhesion molecules

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SUMMARY

Integrins are heterodimeric cell adhesion proteins connecting the extracellular matrix to the cytoskeleton and transmitting signals in both directions. These integrins are suggested to be involved in many different biological processes such as growth, differentiation, migration, and cell death. Of more than 20 known integrins, 10 contain the nearly ubiquitously expressed β1 integrin subunit. Disruption of the β1 integrin gene by homologous recombination allows us to assess the supposed functions of β1 containing integrins in vivo in a new way. This review will present and discuss recent findings derived from such studies concerning the biological roles of β1 integrins in early development, differentiation and migration, hematopoiesis, tumorigenesis, and supramolecular assembly of extracellular matrix proteins. While several former results were confirmed, others were contradicted and new functions found, significantly changing the previous view of β1 integrin function in vivo.

Key words: β1 Integrin, Homologous recombination, Differentiation, Morphogenesis

INTRODUCTION

Integrins are widely expressed cell surface molecules with major roles in a variety of biological processes ranging from cell migration to tissue organisation, growth, differentiation, and programmed death of cells. Furthermore, integrins are involved in processes such as thrombosis, inflammation, and cancer. Integrins are heterodimers consisting of an α and a β subunit. Some of the α and β subunits can associate with several partners. To date, 16 α and 8 β subunits are known forming at least 22 different integrins (Hynes, 1992). The β1 integrin can dimerise with 10 different α subunits (α1–α9, αV). The complexity of the β1 integrin family is further increased by the presence of alternative splicing of mRNAs which occurs in genes encoding α as well as β subunits. In humans the β1 integrin subunit can be expressed in 4 different variants (β1A, β1B, β1C, β1D) which differ in their cytoplasmic domains (van der Flier et al., 1995; Zhidkova et al., 1995; Belkin et al., 1996). β1A is expressed in almost all tissues with the exception of skeletal and cardiac muscle which express β1D. Both the β1B and β1C isoforms are expressed in a few human tissues but have not been found in mouse so far.

Heterodimers of the β1 integrin subfamily bind various components of the extracellular matrix (ECM) including collagens (α1, α2), laminins (α1, α2, α3, α6, α7, α9), fibronectin (α3, α4, α5, α8, αv), vitronectin (αv) but also cell counter receptors such as vascular cell adhesion molecule 1 (VCAM-1) (α4) and fertilin (α6). Although many different β1 integrins can bind to the same ligand each integrin can transduce unique information (Wary et al., 1996). Such different functions of individual α subunits became obvious by gene targeting experiments in mice (Fässler et al., 1996a) where, for example, different fibronectin receptors (α4, α5) produce distinct phenotypes (Yang et al., 1993, 1995). The cytoplasmic domain of β1 integrin can interact with cytoskeletal proteins such as talin and α-actinin (Horwitz et al., 1986; Otey et al., 1990) but also with signaling molecules such as focal adhesion kinase (FAK; Schaller et al., 1995) and integrin-linked kinase (ILK; Hannigan et al., 1996). Among the many signaling mechanisms triggered by β1 integrins are the mitogen-activated protein (MAP) kinase cascade (Schlaepfer et al., 1994; Chen et al., 1994), protein kinase C (PKC; Vuori and Ruoslahti, 1993), phosphatidylinositol hydrolysis (McNamee et al., 1993), Na+/H+ antiporter activity (Schwartz et al., 1994) and, as a very early event, a pathway involving rho (Chong et al., 1994). Intracellular signals, on the other hand, can change the affinity of integrins for their extracellular ligands (Schwartz et al., 1995; Dedhar and Hannigan, 1996). Integrins, therefore, allow bidirectional transfer of information through the cell membrane, from the outside into the cell and vice versa.

What do we know about the in vivo functions of β1 integrins? There is considerable information about the function of integrins in development starting at fertilisation and extending through organogenesis. Antibody perturbation...
experiments identified α6β1 as the receptor for fertilin. The latter is present on sperm cells and crucial for sperm binding and may also play a role during fusion of oocyte and sperm cell membranes (Almeida et al., 1995). In addition, perturbation experiments with antibodies, RGD-containing peptides and anti-sense oligonucleotides demonstrated that β1 integrins are crucial for migration of parietal endoderm on laminin or fibronectin in vitro (Grabel and Watts, 1987; Sutherland et al., 1993) and for migration of neural crest cells (Thiery et al., 1985), myotomal myoblasts (Jaffredo et al., 1988) and neuroblasts (Galileo et al., 1992) in vivo. Finally, antibodies against β1 integrin injected into the blastocoel of salamander Pleurodeles waltlī block gastrulation (Darribere et al., 1988). These findings, however, do not hold in flies where gastrulation occurs normally in the absence of the β1 integrin homologue (Newman and Wright, 1981).

The application of mouse genetics significantly increased our understanding of the in vivo functions of β1 integrins. Mutation of the genes for several α integrin subunits dimerising with β1 integrin resulted in unexpected phenotypes (Fässler et al., 1996a; Hynes, 1996). In the present review we want to focus on results obtained by disrupting the β1 integrin gene in cells and mice. Furthermore, we discuss how these findings have changed our previous view about β1 integrin function.

PRE- AND PERI-IMPLANTATION DEVELOPMENT

The generation of β1-null mice and cells confirmed the importance of β1 integrin for normal cell function and development. A null mutation in the β1 integrin gene gives rise to embryos which develop to the blastocyst stage, initiate implantation but die shortly thereafter (Fässler and Meyer, 1995; Stephens et al., 1995). Although previous in vitro data suggested an early lethal phenotype, several aspects of the phenotype were still unexpected. First, β1 integrin is thought to be required for binding of sperm cells to oocytes (Almeida et al., 1995). Mouse oocytes express high levels of β1 integrin (Fig. 1) which appears on the cell surface in association with α3, α5 and α6 subunits (Hierck et al., 1993; Tarone et al., 1993; Sutherland et al., 1993). Sperm cells express fertilin, which belongs to a class of proteins called ADAMs (a disintegrin and metalloprotease domain; for review see Huovila et al., 1996). Fertilin is composed of two subunits which contain a disintegrin domain and a fusion domain. Elegant in vitro experiments demonstrated that fertilin binds to α6β1 and that antibodies which block β1 or α6 integrin interfere with fertilization (Almeida et al., 1995). The capability of eggs carrying a null mutation in the β1 integrin gene to become efficiently fertilized is clearly due to the presence of maternal mRNA coding for β1 and α6 subunits. Gene transcription is halted during meiotic divisions of germ cells and the pool of mRNA transcribed before is equally divided among daughter cells (Stebbins-Boaz and Richter, 1997). As a consequence, oocytes will have β1 integrin mRNA although they carry at the same time a null mutation in their haploid genome. Therefore, fertilization also occurs normally in embryos carrying a null mutation in the α6 integrin gene (Georges-Labouesse et al., 1996).

The normal preimplantation development of β1-null embryos is remarkable since members of the β1 integrin family and several of their ligands are expressed during this early period of development (Sutherland et al., 1993; Damsky et al., 1993). Again, the presence of maternal mRNA could be an explanation for the lack of phenotype although immunostaining of β1-null embryos and northern blot data of normal embryos suggest that the maternal β1 integrin mRNA disappears soon after fertilisation (Fig. 1). Alternatively, αv integrins may compensate for the absence of the β1 integrin during the preimplantation period. Expression studies have shown that at least two αv complexes are expressed from fertilisation onward (Sutherland et al., 1993). Finally, it is possible that β1 integrins play a dispensable role during the development from a one cell embryo to a blastocyst. This conclusion is supported by our northern blot results which show detectable amounts of embryo-derived β1 mRNA only at a late preimplantation stage, in compacted morulae and blastocysts (Fig. 1).

Another surprising finding was that β1-null embryos are able to initiate implantation which suggests that trophoderm formation as well as trophoblast differentiation and function are β1 integrin-independent (Fässler et al., 1995; Stephens et al., 1995). The differentiation of trophoderm into trophoblast occurs in blastocysts shortly before implantation and is characterized by a conversion of the external surface of the embryo from a quiescent, nonadhesive to a protrusive, adhesive phenotype (Sutherland et al., 1988). Clearly, β1 integrin is not necessary for this conversion and for the attachment of β1-null embryos to the uterine epithelium in vivo. Moreover, β1-null embryos are also able to degrade the basement membrane and invade the uterine stroma. This finding was unexpected, since penetration of tumor cells through the amniotic basement membrane, a pathological but related process, could be inhibited by RGD-containing peptides (Gehlsen et al., 1988). In contrast to the normal trophoblast differentiation, β1-null embryos fail to form normal extraembryonic endoderm leading
to a rapid deterioration of ICM in vitro (Stephens et al., 1995). Also in vivo, the ICM of β1-null mice deteriorates rapidly, always prior to the trophoblast (Fässler and Meyer, 1995). The reason for the ICM failure is not entirely clear but a defect in the formation or the survival of visceral and parietal endoderm is most likely. First, in vitro outgrowths of β1-null blastocysts provided clear evidence that the morphogenesis of endoderm occurs only in a few embryos, limited to only a few cells (Stephens et al., 1995). Second, β1-null ES cells contribute to all three germ layers after injection into normal blastocysts although β1-null endodermal cells are only rarely observed (Fässler and Meyer, 1995). In addition, detailed analyses of mature tissues from β1-null chimeric mice revealed that mutant cells are always absent from endodermal derived cell lineages (J. Schittny and R. Fässler, unpublished observations). Third, the survival of embryonic cells during caviation processes is dependent on survival signals triggered by interactions of cells with the basement membranes (Coucouvanis and Martin, 1995). The first basement membrane formation is observed in adhesive competent blastocysts between the primitive endoderm and ICM cells. Since β1-null embryos do not form proper basement membranes, endodermal cells would die and constantly be replaced until the ICM is exhausted. Preliminary studies suggest that programmed cell death occurs in deteriorating β1-null blastocysts.

Lethal phenotypes occurring as early as in the β1-null mice can provide a wealth of new information, but still leave many questions unanswered. Can mutant cells migrate during development? Do mutant cells participate in organogenesis and cell lineage differentiation? Do subtle alterations occur in differentiated null cells? Such questions can be answered if genes are conditionally inactivated at a certain time or in a certain tissue using the cre/loxP system (Gu et al., 1994). An alternative is to generate ES cells with null mutations in both alleles and to inject them into normal blastocysts. Such β1-null chimeric mice proved to be a valuable tool for studying the role of β1 integrin in cell migration, survival and differentiation in vivo.

DIFFERENTIATION AND MIGRATION

During differentiation cells are exposed to a continuously changing environment which includes alterations in the composition of the ECM and/or interactions with new neighbouring cells. The new cellular environments together with newly acquired functions are often accompanied with changes in integrin expression and avidity. During myogenesis, for example, all members of the β1 integrin subfamily are expressed. The expression of some integrins such as α4β1 and α7β1, however, is highly regulated and occurs only at very specific time points during differentiation (Rosen et al., 1992; Velling et al., 1996; Martin et al., 1996). Alterations in integrin avidity range from a decrease to a complete loss of integrin function and depend on elicitation of intracellular signaling mechanisms which are generally called ‘inside-out signaling’ (Schwartz et al., 1995; Dedhar and Hannigan, 1996). Evidence for a modulation of integrin avidity without a corresponding change in levels of integrin expression comes from blood cells (Alon et al., 1995; Berlin et al., 1995) and retinal neurons (Neugebauer and Reichardt, 1991). Retinal neurons, for example, continuously express laminin-binding integrins but attach and extend neurites on laminin only during early and late, but not intermediate stages of development (Neugebauer and Reichardt, 1991). In addition to the fine tuning of integrin function during cellular differentiation, antibody perturbation experiments have provided direct evidence for an important role of β1 integrin during differentiation of a large number of cell types including osteoblasts (Moursi et al., 1996), mammary epithelial cells (Boudreau et al., 1995) and myoblasts (Menko and Boettiger, 1987).

The availability of β1-null ES cells made it possible to directly test the role of β1 integrin in differentiation by a variety of means. β1-null ES cells can be aggregated in vitro to form so-called embryoid bodies which contain well differentiated cells derived from all germ layers (Doetschman et al., 1985). Alternatively, β1-null ES cells can be differentiated in vivo after injection into syngeneic mice where they form teratomas which are benign, locally growing tumors (Damjanov and Solter, 1974, 1976). Finally, β1-null ES cells can be injected into normal blastocysts which are then transferred into foster mice resulting in chimeric animals. The production of such β1-null chimeric mice allows us to determine whether β1-null ES cells contribute to mature tissues and whether they can reach a fully differentiated state. The results of all these experiments, which will be described here and in the following sections, have demonstrated that some suggested functions of β1 integrin have passed and others have failed the genetic test.

The disruption of both β1 integrin alleles in ES cells is accompanied by an altered adhesion to and an impaired ability to migrate on ECM proteins (Fässler et al., 1995). Similar results were also obtained with β1-null F9 cells (Stephens et al., 1993). In addition, β1-null ES cells lose their tight cell-cell contacts, become round and acquire microvilli-like structures. These changes, however, are not associated with an altered expression or deposition of cytoskeletal proteins. Despite these gross deficits in vitro, β1-null ES cells can readily integrate themselves into the inner cell mass of blastocysts and contribute to most ectodermal and mesodermal lineages (Fässler et al., 1995; Fässler and Meyer, 1995). However, they are rarely found in the endoderm of early embryos and are never present in mature endoderm-derived lineages such as liver, gut or lung epithelial cells (J. Schittny and R. Fässler, unpublished observation). These results are supported by findings obtained from in vitro differentiated β1-null F9 embryonal carcinoma cells, where migration and epithelialization of endoderm is defective (Stephens et al., 1993). The number of β1-null ES cells that contribute to the developing mouse is critical: while extensive contribution results in highly abnormal tissue formation and interference with embryo survival, low contribution does not interfere with development and gives rise to normal β1-null chimeric mice containing β1-null cells with well differentiated phenotypes in many mature tissues (Fässler and Meyer, 1995). Biochemical and histochemical analyses of β1-null chimeric mice revealed a number of interesting results: hematopoietic organs such as bone marrow, thymus, spleen and blood lack β1-null cells (see next section), the contribution to most other tissues is low, and many highly migratory cells including myoblasts, neural crest cells and neurons can be detected in mature tissues. The latter finding was unexpected since antibodies against β1 integrin and RGD-containing peptides or β1 integrin antisense mRNA can inhibit the migration of all
three cell types (Thiery et al., 1985; Jaffredo et al., 1988, Galileo et al., 1992). One explanation for the different results could be the different assay systems employed: genetic ablation of β1 integrin in totipotent ES cells may activate compensatory pathways which are silent in differentiated cells. Another explanation could be that the presence of a mixed cell population in β1-null chimeric mice allows null cells to migrate passively by interacting with wild-type cells. Finally, RGD-containing peptides or antisense mRNAs may inhibit multiple integrin subfamilies. Several integrins bind RGD motifs and all β subunits have a rather high degree of sequence similarity which could allow an antisense mRNA of β1 integrin to inactivate a broad range of β subunits. Such scenarios may account for findings which suggest that a 50% reduction of β1 integrin expression in chick neuroblasts inhibits migration (Galileo et al., 1992) whereas mice heterozygous for a β1-null mutation display no defect in migration of neuroblasts or other highly migratory cells.

Although embryo survival is associated with low contribution of β1-null cells to mature tissues, skin and skeletal muscle of adult β1-null chimeric mice often contain large numbers of β1 integrin-deficient cells. The formation of both tissues, skin and muscle was thought to depend on β1 integrin. Keratinocytes, for example, express several β1 integrins (α2β1, α3β1 and α5β1) which are normally confined to the basal layer of keratinocytes (Jones and Watt, 1993; Watt and Hertle, 1994). Experiments with cultured keratinocytes suggested that β1 integrins mediate adhesion and migration and in addition, regulate stratification and the initiation of terminal differentiation (Jones et al., 1995). Interestingly, a defect in differentiation is observed when β1-null ES cells are differentiated into keratinocytes in vitro via embryoid bodies (Bagutti et al., 1996). When β1-null ES cells are differentiated in vivo such as in β1-null teratomas or chimeras, however, differentiation of keratinocytes occurs normally (Bagutti et al., 1996). The discrepancy between the in vitro and the in vivo results can be due to a defect in formation of basement membrane (see below) which is abnormal in vitro but is still, at least partly, maintained in vivo by wild-type cells which are present in the surrounding stromal tissues of both teratomas and chimeras. Alternatively, growth factors present in vivo but absent in vitro can gain important roles when integrins are absent.

Also, during formation of skeletal muscle several important events including migration of myogenic cells from the somites to their peripheral targets (Jaffredo et al., 1988), withdrawal from the cell cycle and differentiation (Menko and Boettiger, 1987; Sastry et al., 1996), formation of syncytial myotubes (Rosen et al., 1992) and assembly of sarcomeres (Volk et al., 1990). When β1-null ES cells are differentiated in vivo such as in β1-null teratomas or chimeras, however, differentiation of keratinocytes occurs normally (Bagutti et al., 1996). The discrepancy between the in vitro and the in vivo results can be due to a defect in formation of basement membrane (see below) which is abnormal in vitro but is still, at least partly, maintained in vivo by wild-type cells which are present in the surrounding stromal tissues of both teratomas and chimeras. Alternatively, growth factors present in vivo but absent in vitro can gain important roles when integrins are absent.

HEMATOPOIESIS

During hematopoiesis, hematopoietic precursor cells (or stem cells) originate from so-called hemangioblasts which are common precursors for blood as well as endothelial cells. Such hemangioblasts differentiate at intraembryonic sites, in the aorta-gonad-mesonephros (AGM) region (Medvinisky and Dzierzak, 1996; Cumano et al., 1996), and at extraembryonic sites, in the yolk sac tissue (Russell and Bernstein, 1966). At around embryonic day 9, when blood circulation begins, precursor cells from both regions enter the blood and settle
approximately one day later in the fetal liver (Fig. 3). β1-null precursor cells are present in AGM, yolk sac, and in the fetal blood but never in the fetal liver (Hirsch et al., 1996; A. Potocnik, K. Eichmann and R. Fässler, unpublished). β1-null precursor cells are also absent from fetal thymus, fetal spleen or bone marrow. This could be a consequence of a defect in colonising to the fetal liver, which might be essential for blood cell development (Fig. 3). Alternatively or in addition, absence of β1 integrin might abolish direct homing of hematopoietic stem cells from the blood to these organs. The mechanism which underlies the inability to settle in the fetal liver is not known. Interactions of precursor cells with endothelial cells via α4β1 and VCAM-1 cannot account for the defect since hematopoietic precursors lacking the α4 subunit can home to the fetal liver and into all other hematopoietic organs (Arroyo et al., 1996). Interestingly, however, the absence of α4 integrin results in a defect in T- and B-cell development. In α4-null chimeric mice, B-cell differentiation in the bone marrow is blocked before the pro-B-cell stage. This defect is not observed when β1-null ES cells are differentiated in vitro indicating that αβ7 integrin, which is still expressed in the absence of β1 integrin, may be sufficient for normal B-cell differentiation (Hirsch et al., 1996). The T-cell defect in α4-null chimeric mice includes an abnormal differentiation of T-cell precursors in the bone marrow and a defective homing of T-cells to Peyer’s patches. The lack of T-cells in Peyer’s patches is mainly a consequence of a loss of αβ7 since β7-null mice show an 80-90% reduction of lymphocyte migration to the Peyer’s patches (Wagner et al., 1996). The abnormal differentiation of α4-null T cells resides in the bone marrow. Early T-cell precursor can develop in the fetal liver and home to the fetal thymus and, as a consequence, α4-null mice have for the first month after birth normal numbers of T cells. When these mice age, however, T-cells decrease in number. Elegant cell transfer experiments have shown that the α4-null pre T-cells develop in bone marrow but never leave to enter into the circulation.

Antibody perturbation experiments have suggested that α6β1 integrin plays a role in the homing of pre T-cells to the thymus (Ruiz et al., 1995). Preliminary analyses of mice lacking the α6 subunit (Georges-Labouesse et al., 1996) suggest, however, that neither the development of the thymic anlage nor homing of T-cells to thymic tissue is abnormal (E. Georges-Labouesse, personal communication). Although this finding indicates that homing can occur in the absence of α6 integrin it is not known yet whether differentiation of α6-null T-cells is defective in these animals.

**Fig. 2.** β1 integrin and skeletal muscle development. Broken lines indicate steps in skeletal myogenesis which have been suggested as β1 integrin-dependent by antibody perturbation assays (for detail see text). Characteristic stages of myogenesis are indicated and refer to the development of the mouse.

**Fig. 3.** β1 integrin and hematopoiesis. Schematic presentation of migration of blood cell precursors during development. The broken lines indicate integrin-dependent migration or differentiation events (for detail see text). Characteristic stages of hematopoiesis are indicated and refer to the development of the mouse.
TUMORIGENESIS

During the last few years a number of reports have demonstrated that integrins influence tumorigenicity ranging from local tumor growth to metastasis (Varner and Cheresh, 1996). On the other hand, however, it has become clear that tumorigenicity is not regulated by ‘specific tumor integrin(s)’. Therefore, it is not surprising that some studies link tumorigenicity to a reduced expression of β1 integrin and others to an elevated expression. Some breast cancer cell lines or Chinese hamster ovary (CHO) cells, for example, lack α2β1 or α5β1 integrin function, respectively. The malignant phenotype of these cell lines could be reduced by overexpression of integrin α subunits (Zutter et al., 1995; Giancotti and Ruoslahti, 1990). Contrary to these findings, another report provides evidence that perturbing β1 integrin function with specific antibodies decreases the malignant phenotype thus correlating β1 integrin expression with malignancy (Weaver et al., 1997). The seemingly controversial results suggest that the influence of an altered level of a given integrin on tumorigenicity is dependent upon cell specific parameters. One of them might be the relative expression levels of different integrins. In addition, tumor cells originate from many tissues which have a different ECM. Hence, it is not surprising that certain integrins which play important roles in one tumor have no role in another tumor type when the respective ligands are absent in the tumor stroma.

To test how the absence of the entire β1 integrin subfamily influences the growth of tumors, teratomas were generated with normal and β1 integrin-deficient ES cells (Bloch et al., 1997). Teratomas are benign tumors which originate from totipotent cells such as germ cells or ES cells (Damjanov and Solter, 1974, 1976). They contain derivatives of all three germ layers, grow locally and do not seed into distant organs. In the absence of β1 integrin teratoma growth is drastically impaired suggesting a growth promoting role of β1 integrin in this type of tumor. Alterations in β1-null teratomas range from increased apoptosis to abnormal deposition of ECM proteins and a defect in angiogenesis. An increase in cell death may result from the fact that teratomas are composed of normal cells which still need anchorage for survival. It is known that normal cells will undergo apoptosis when deprived of their interaction with the surrounding matrix (Ruoslaihti and Reed, 1994). The molecular mechanism of integrin-mediated cell survival is still poorly understood. Certain integrins can induce bcl-2 expression which is a well known suppressor of apoptosis (Zhang et al., 1995). Other integrins activate via Shc the MAP kinase pathway (Wary et al., 1996). Since inhibition of MAP kinase can induce apoptosis (Xia et al., 1995), integrin mediated elevation of MAP kinase activity conceivably could promote cell survival.

From studies with integrin-null cells and mice (see next section) it is known that integrins modulate the supramolecular organisation of ECM proteins. Teratoma cells produce and deposit an ECM in a very characteristic fashion (Bloch et al., 1997). Matrix proteins such as collagens and fibronectin are present in cord-like structures always along cells with high β1 integrin expression. In addition, basement membranes are present and tightly attached to epithelial cells. β1-null teratomas are different. Although they produce all ECM proteins which have been found in normal teratomas the structure of the ECM is abnormal. Collagens and fibronectin are diffusely deposited and rarely concentrated in cord-like structures. Basement membranes form but detach from cells. In addition, some basement membrane constituents such as laminin-1 and nidogen/entactin are reduced in β1-null tumors (Sasaki et al., in press). The reduction of laminin-1 is due to a diminished production while nidogen/entactin becomes proteolytically degraded. Such qualitative and quantitative changes of the ECM may contribute to the reduced teratoma growth by affecting the binding of growth factors, expression of growth factors and growth factor receptors, and by diminishing direct mitogenic activity of certain ECM components (Adams and Watt, 1993).

The most interesting change in β1-null teratomas is their defect in angiogenesis. Normal ES cells can readily differentiate into endothelial cells both in vitro and in vivo. β1-null ES cells, however, can differentiate in vitro but are never found as endothelial cells in teratomas (Bloch et al., 1997). Similarly, they are also absent from organs of β1-null chimeric mice such as liver (Fässler and Meyer, 1995). The absence of β1-null endothelial cells in vivo might be due to their diminished ability to initiate vessel formation which is clearly evident from in vitro experiments. While normal and β1-null endothelial cells differentiate equally well, the formation of vessels is enormously delayed with β1-null cells. Such a delay is at least partially compensated for in vivo by normal endothelial cells and as a consequence mutant cells are never found in organs of β1-null chimeric mice or teratomas. This influence of β1 integrin on angiogenesis was unexpected, since previous antibody perturbation experiments identified αvβ3 and αvβ5 as important mediators of angiogenesis while antibodies against β1 integrin were ineffective in these studies (Brooks et al., 1994a,b; Friedlander et al., 1995). A reduced expression of αvβ3 and αvβ5 integrin in β1-null embryoid bodies cannot explain the delay in vasculogenesis because both are expressed at normal levels. An explanation for this discrepancy may be an incomplete inhibition of β1 integrin function with a single injection of antibodies, especially if one takes into account that virtually all cells express β1 integrin and hence might function as a sink.

Several growth factors were shown to exert their angiogenic signal in concert with endothelial integrins (Friedlander et al., 1995). Vascular endothelial growth factor (VEGF) which effectively induces proliferation of endothelial cells and branching of blood vessels, was found to depend on αvβ5 integrin. Similarly, in embryoid bodies lacking β1 integrin function VEGF neither stimulates endothelial cell proliferation nor sprouting of blood vessels. This finding clearly demonstrates that VEGF activity is dependent on β1 integrin function (Bloch et al., 1997).

An important aspect of the involvement of β1 integrin in angiogenesis is the potential implication for tumor treatment. Tumors depend largely on effective angiogenesis (Hanahan and Folkman, 1996). Several proteins have been identified in the past which block tumor angiogenesis and as a consequence tumor growth. Future studies will show whether β1 integrin blocking substances will fall in line with other promising factors that efficiently interfere with tumor angiogenesis.

While tumor growth is differently modulated by β1 integrin, metastasis is in most cases promoted. Antibodies against β1 (Elliott et al., 1994), α5β1 (Newton et al., 1995) and α6...
integrin (Ruiz et al., 1993) and RGD peptides (Humphries et al., 1988) reduce metastasis in different in vivo models. Tumor invasion requires tumor cells to adhere to ECM components and basement membranes (Stetler-Stevenson et al., 1993). This adhesion event triggers the release of proteases which allows cancer cells to invade the surrounding ECM and to cross basement membranes. In vivo studies with β1-deficient tumor cells confirmed the importance of β1 integrin for metastasis but demonstrated further, that β1 integrin is not essential for metastasis. Cells from a ras-myc transformed fibroblastoid tumor cell line lacking β1 integrin gene expression form solid tumors and small lung metastases after injection into the tail vein (C. Brakebusch et al., unpublished). Transfection of these cells with a β1 integrin cDNA resulted in a clonal cell line that forms tumors with a similar growth rate, but much larger lung and, in addition, small liver metastases. Similar results were obtained with the murine T-cell lymphoma cell line ESb (P. Stroeken et al., unpublished). After disruption of both β1 integrin alleles by homologous recombination, invasion of β1-null ESb cells into lungs is reduced which leads to an increased survival rate of injected animals. Interestingly, however, β1-null ESb cells invade muscle tissues and meninges which is not the case if they express β1 integrin. The results of these experiments suggest that ablation of β1 integrin expression may alter the adhesive ‘finger-print’ of tumor cells leading to a change in the homing to metastatic target tissues.

SUPRAMOLECULAR ASSEMBLY OF ECM PROTEINS

Extracellular matrix molecules form either homophilic or heterophilic interactions resulting in homo- (such as fibronectin, collagens, etc.) or heteropolymeric complexes (such as basement membranes) of defined chemical composition and supramolecular architecture (McDonald, 1994). In many cases matrix assembly can be replicated in vitro. In other cases the assembly requires cells and occurs at the cell surface. Fibronectin, for example, can only assemble into a disulfide-linked pericellular network if cells are present which express activated integrins. Several reports identified α5β1 as the first integrin which is able to mediate fibronectin polymerisation (Mosher et al., 1992). Transfection of α5 integrin into CHO cells leads to a large increase in fibronectin assembly (Giancotti and Ruoslahti, 1990; Wu et al., 1993). In contrast, antibodies to α5 and β1 (Akiyama et al., 1989) and dominant-negative mutations in β1 integrin inhibit fibronectin assembly (LaFlamme et al., 1994). With the isolation and analysis of fibroblasts derived from mice lacking the α5 integrin gene it became clear that other integrin(s) or non-integrin receptors can also assemble fibronectin (Yang et al., 1993). This was confirmed by several recent findings. A β1-null fibroblast cell line (called GD25) can bind to fibronectin and assemble a fibronectin matrix (Wennerberg et al., 1996). Immunohistochemical studies colocallized αv and β3 integrin with the fibronectin fibrils suggesting that αvβ3 mediates fibronectin polymerization. Interestingly, many more and much better developed fibrils are deposited when GD25 cells are transfected with a wild-type β1 integrin cDNA and express α5β1 on the cell surface. Furthermore, in transfected GD25 cells α5β1A integrin dominates the polymerisation process while αvβ3 integrin is mediating attachment at the focal contacts. The biological reason for this differential usage of integrins is not known, but it may play a role in late stages of development, in certain stress situations, or in diseases, which cannot be tested in α5 or β1 integrin knockouts due to the early lethal phenotype. In another experiment, expression of activated forms of αIIbβ3 allowed α5-deficient CHO cells to assemble a fibronectin matrix (Wu et al., 1995; Hughes et al., 1996). Although αIIbβ3 probably never serves this function in vivo, these data are remarkable and suggest that all fibronectin-binding integrins can assemble a fibronectin matrix when they are activated and interact with an intact actin cytoskeleton. This hypothesis is supported by results obtained with α5-null fibroblasts and α5-deficient CHO cells. Whereas αvβ1 functionally compensates for the lack of the fibronectin receptors in α5-null fibroblasts and allows these cells both to adhere to and assemble fibronectin (Yang and Hynes, 1996), overexpression of αv in α5-deficient CHO cells promotes only adhesion to but not assembly of fibronectin (Zhang et al., 1993). Clearly, adhesion is not sufficient for CHO cells to polymerize fibronectin and what may be lacking in these cells is a proper interaction with cytoskeletal proteins.

Basement membranes are abundant specialized extracellular matrices localised in close vicinity to cells (Timpl and Brown, 1996; Streuli, 1996). Major components of basement membranes are various forms of collagen IV and laminin, proteoglycans and small glycoproteins such as nidogen/entactin. Experimental evidence obtained from in vitro studies has suggested that basement membrane formation is to a great extent a self-assembly process (Yurchenco and O’Rear, 1994). One hypothesis suggests that laminins and collagen IV are forming independent network-like polymers which are bridged by nidogen (Fox et al., 1991). Evidence for a central role for nidogen in basement membrane assembly comes from studies which show that it interacts not only with laminin but also with several other components including perlecan and collagen type IV. The first report which associated basement membrane formation in vivo with integrin function came from studies of α3-null mice (Kreidberg et al., 1996). Kidneys of these mice have fragmented and disorganized glomerular basement membranes suggesting that either the formation or the maintenance of these membranes are defective. Additional defects include the lack of foot processes from podocytes and a reduction in the number and enlargement of glomerular capillary loops. Similar defects in basement membrane assembly were observed in β1-null blastocysts (Stephens et al., 1995), in differentiated β1-null F9 cells (Stephens et al., 1993) and in teratomas derived from β1-null ES cells (Bloch et al., 1997). β1-null blastocysts or visceral endoderm cells derived from β1-null F9 cells produce laminin but fail to deposit it into a linear basement membrane (Stephens et al., 1993, 1995). In β1-null teratomas basement membranes form, but display morphological and biochemical alterations (Bloch et al., 1997). Morphologically, the lamina lucida is irregularly enlarged and the basement membrane is partially or completely detached from the cell surface. Partial detachment leads to the formation of loops, which can result in large bubble-like structures. Biochemical analyses revealed that in β1-null teratomas the content of laminin-1 is decreased by approximately 90% which is mainly due to a downregulation of the mRNA transcription of individual laminin subunits (Sasaki et al., in press).
Expression of nidogen is reduced by approximately 70%, which is due to a decreased half-life of unbound nidogen. Expression levels of other basement membrane components such as perlecan, fibulin-1, fibulin-2, and fibronectin are not altered. This finding together with the fragmented glomerular basement membranes in α3-null mice, suggests that β1 integrins are involved in either actively organizing or, at least, in maintaining the structural integrity of basement membranes.

The role of β1 integrins in the assembly and maintenance of ECM matrices has significant implications. First, basement membranes are reservoirs for growth factors (Adams and Watt, 1993) and intimate contact with cell surfaces may be crucial for appropriate interactions with relevant receptors. Second, intimate contact between cells and basement membranes may be necessary for cell polarization and hence for cellular function. Third, a number of ECM proteins bind to matrices which are assembled by integrins. Defects in ECM assembly could consequently also result in abnormal deposition of molecules such as fibulin-1, fibulin-2, collagens, etc.

PERSPECTIVES FOR THE FUTURE

The last few years have witnessed considerable progress in the field of integrin research. The generation of mutant mouse strains has rapidly advanced the field (Fässler et al., 1996a; Hynes, 1996). Results from these studies have provided new insights into integrin function, and have sometimes supported but more often contradicted earlier conclusions obtained from antibody and peptide inhibition studies. Similar contradictions have also been observed after targeted disruption of other genes (Saga et al., 1992; Forsberg et al., 1996). Inhibition of protein function by the genetic route and by antibodies or peptides has specific advantages and disadvantages. The advantages of genetically introduced null mutations into cells and mice include the complete and stable elimination of protein function which may not always be the case with antibodies. The genetic approach has no or few side effects. This is different, for example, for antisense oligonucleotides which can have unspecific cytotoxic effects (Durbeej et al., 1993), or antibodies which can exert stimulatory instead of blocking activity. The disadvantages of knockout mice is that constitutive gene disruptions may not always produce a phenotype because other proteins with overlapping functions can fulfill the task of the deleted proteins. This functional redundancy has been elegantly demonstrated for muscle-specific transcription factors or the engrailed genes (Wang et al., 1996; Hanks et al., 1995). Alternatively, the lack of phenotype in genetic approaches and the severe defects with function-interfering antibodies or peptides can be explained with ‘compensation’, i.e. the knockout-specific activation or upregulation of functionally compensating proteins during development. One has to keep in mind, however, that such a compensation has not been demonstrated so far and it is up to future experiments to test whether ‘compensation’ exists. Early lethal phenotypes, as is the case for β1 integrin are other disadvantages of knockouts. The death of the embryo prevents all functional studies of later developmental stages, leaving many questions concerning the in vivo function of a protein unanswered. Establishment of β1-null ES cells and differentiation of these cells in chimeric mice, embryoid bodies and teratomas can be applied to address some of these questions. Other ways of tackling the remaining problems are ‘knocks’ where one gene is replaced by another (for example replacing a β1 integrin gene with a gene containing subtle mutations or by a β3 integrin gene), inducible knockouts and knockins, where gene inactivation or replacement occurs in a tissue- or time-specific manner, or multiple knockouts, where mice with several null mutations are generated by crossing knockout mice. The use of these techniques and their refinements will surely overcome most shortcomings of constitutive knockouts and will allow a more detailed understanding of β1 integrin function in vivo.

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