Integrins as receptor targets for neurological disorders

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ABSTRACT

This review focuses on the neurobiology of integrins, pathophysiological roles of integrins in neuroplasticity and nervous system disorders, and therapeutic implications of integrins as potential drug targets and possible delivery pathways. Neuroplasticity is a central phenomenon in many neurological conditions such as seizures, trauma, and traumatic brain injury. During the course of many brain diseases, in addition to intracellular compartment changes, alterations in non-cell compartments such as extracellular matrix (ECM) are recognized as an essential process in forming and reorganizing neural connections. Integrins are heterodimeric transmembrane receptors that mediate cell–ECM and cell–cell adhesion events. Although the mechanisms of neuroplasticity remain unclear, it has been suggested that integrins undergo plasticity including clustering through interactions with ECM proteins, modulating ion channels, intracellular Ca2+ and protein kinase signaling, and reorganization of cytoskeletal filaments. As cell surface receptors, integrins are central to the pathophysiology of many brain diseases, such as epilepsy, and are potential targets for the development of new drugs for neurological disorders.

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1. Introduction

Neuroplasticity is the ability of the nervous system to react with adaptive changes to intrinsic or extrinsic challenges such as epilepsy, trauma, and brain injury. In many cerebrovascular and neuronal diseases, in addition to intracellular compartment changes, alterations in non-cell compartments such as extracellular matrix (ECM) are recognized as an essential process. There has been recent interest in the possible role of adhesion molecules, particularly integrins as ECM receptors, in neurological disorders because they form an important link between the ECM and the intracellular cytoskeleton (CSK) and signaling molecules (Fig. 1). In the brain, ECM proteins are synthesized and secreted into the extracellular space in a mesh-like structure by neurons and glial cells. During development and mature nervous systems, and in wound healing, the interaction between ECM and its receptor integrin has a pivotal role in maintaining structural and...
Integrins are necessary for neuronal cells to attach, spread, migrate, and extend processes on ECM molecules. Interactions of ECM–integrin–focal adhesion complex (FAC) including CSK proteins and intracellular signaling molecules in neuronal systems play an important role in synaptic morphology and number, neuron–neuron and neuron–muscular synaptic transmission, and neuroplasticity that modulates neuronal cell proliferation, migration, and differentiation (Venstrom & Reichardt, 1993). Historically, the role of the ECM and its receptors was viewed as largely structural molecules. However, there is emerging evidence that these proteins and receptors also perform signaling and regulatory functions in neuronal pathophysiological processes such as memory, inflammation, wound healing, epileptogenesis, angiogenesis, and tumor metastasis. Therefore, integrins as cell surface receptors are attractive pharmacological targets for designing new therapies for brain diseases (Horwitz, 1997; Clemetson & Clemetson, 1998; Wu & Davis, 1998; Davis et al., 2001; Ross & Borg, 2001; Gall & Lynch, 2004; Jin & Varner, 2004; Lal et al., 2007; Cox et al., 2010; Millard et al., 2011; Reichardt & Prokop, 2011).

This review describes the neurobiology of integrins, pathophysiological roles of integrins in neuroplasticity, and the therapeutic implications of integrin targeted drugs for nervous system disorders.

2. Structure and function of integrins

2.1. Integrins in neurons

Integrins are αβ-heterodimeric transmembrane glycoprotein receptors that mediate cell–ECM and cell–cell adhesion events through functional neuroplasticity. ECM and integrin aberrations are likely to contribute to imbalanced synaptic function in epilepsy, Alzheimer’s disease, mental retardation, schizophrenia and other conditions in the brain. (Dityatev & Schachner, 2003; Gall et al., 2003; Gall & Lynch, 2004; Dityatev et al., 2010).

Studies of integrin-initiated intracellular signaling have shown that integrins modulate Ca2+ and K+ ion channels, intracellular Ca2+ concentrations, cellular contractile properties, protein kinase activity, and growth factor receptors (Hynes, 1992; Yip & Marsh, 1997; Porter & Hogg, 1998; Wu et al., 1998; Barouch et al., 2000; Chan et al., 2001; Danen & Yamada, 2001; Davis et al., 2001; Davis et al., 2002; Waitskus-Edwards et al., 2002; Martinez-Lemus et al., 2003; Ruecksschloß & Isenberg, 2004; Gui et al., 2006; Wu et al., 2008a, 2008b, 2010b, 2010c; Yang et al., 2010; Wu et al., 2011a). Furthermore, ECM–integrin–CSK interactions play crucial roles in gene expression, cell proliferation, migration and differentiation, and cell survival (Hynes, 1992; Yamada & Miyamoto, 1995; Schwartz, 2001; Kim et al., 2011).

Since the discovery of the first integrin receptor for ECM protein fibronectin (FN) in 1986 (Tamkun et al., 1986), eighteen α-subunits and eight β-subunits have been identified (Hynes, 2002). Interestingly, there are at least 24 distinct integrins (Reichardt & Prokop, 2011) even though there are twenty-four α-subunit and nine β-subunit genes in the human genome (Venter et al., 2001). A total of over 50,000 papers are published on integrins so far. Fewer than 1000 papers are in the field of neuroscience as compared to ~6000 papers in the field of neurobiology as compared to ~6000 papers in the field of neuroscience as compared to ~6000 papers.
binding ECM proteins such as fibronectin (FN) and transmembrane proteins such as neural cell adhesion molecule (NCAM) on adjacent cells. Integrins also have functional relationships with other membrane receptors such as ion channels and growth factor receptors. Integrins are expressed by many cell types, including neuronal cells, leukocytes, tumor cells, cardiac cells, skeletal muscle cells, and vascular cells (Table 1 and Fig. 1). The α-subunit determines integrin ligand specificity, and the β-subunit is connected to CSK and intracellular molecules and thereby affecting multiple signaling pathways. The extracellular domains interact and bind to ECM in a divalent cation-dependent manner. Short cytoplasmic tails of integrin associate with FAC such as CSK proteins and protein kinases such as non-receptor tyrosine kinases (NRTK) (Wang et al., 1993; Davis et al., 2001).

Integrins are distributed ubiquitously with increased expression in the axon growth cone in the central nervous system (CNS). They are highly differentiated in the brain with region-specific and cell type-specific expression (Fig. 2). The β1-integrin receptors were first identified as mediators of neurite outgrowth on ECM components (Reichardt & Tomaselli, 1991). Since then at least 14 of the 24 known integrin subunit combinations have been reported in nervous system: α1(1), α2(1), α3(1), α4(1), α5(1), α6(1), α7(1), α8(1), α9(1), α10(1), α11(1), α12(1), α13(1), and α14(1) (Table 1 and Fig. 2) (Pinkstaff et al., 1999; Renaudin et al., 1999; Yanagida et al., 1999; Chun et al., 2001; Chan et al., 2003; Morini & Becchetti, 2010). Different heterodimers of integrin have different affinities to the various ECM ligands and will be activated according to ligand and generate a transient or persistent signals. The predominance of α1(1), α2(1), α4(1), α5(1), α6(1), α7(1), and α9(1) integrins in neurons suggest there are extensive interactions with collagens (CN), FN, laminin (LN), and vitronectin (VN). A single integrin heterodimer can often bind more than one ligand and vice versa. For instance, α3(1) can bind to CN, FN, and LN whereas at least 4 heterodimers containing the β1 subunit alone, α2(1), α4(1), α5(1), α5(1), and α9(1) have been identified as FN receptors (Table 1).

Some integrins will increase expression in specific physiological and pathological situations. α1,-, α3,-, and α4-integrin subunits are widely distributed in the developing brain, while α5- and α7-integrin subunits are distributed in the adult brain (Pinkstaff et al., 1999). Immunohistochemistry studies show that α1, α2, α4, α5, α7,-, p53,-, and p4-integrins are increased in the hippocampus in rats with pilocarpine-treated epilepsy (Fasen et al., 2003).

2.2. Integrins in glial cells

In general, integrins control cell migration, proliferation, and differentiation in non-neuronal glial cells. Glial cells play a key role, via integrins, in the development and repair of the nervous system by providing scaffolds and nutrition for (migrating) neurons from astrocytes and radial glia, myelination for ensheathing neurons from Schwann cells in peripheral nervous system (PNS) and oligodendrocytes in CNS, and neuroimmunological protection from microglia. At least 16 of the 24 known integrin subunit combinations have been reported in glial cells (Table 1).

Different integrins show diverse function in glial cells. Integrin β1-subunit and reelin are required for normal morphological formation of radial glial cells and Schwann cell differentiation (Forster et al., 2002; Pietri et al., 2004). The α5-integrin is essential for glial proliferation and the α4-integrin contributes for survival. The loss of α4-integrin or α5-integrin did not affect glial cell migration and differentiation (Haack & Hynes, 2001). In addition, αβ3-integrins mediate oligodendrocyte migration, αβ3 integrin facilitates oligodendrocyte proliferation and Schwann cell adhesion and migration, αβ1 integrin is involved in remodeling in Schwann cells, αβ3 integrin allows astrocytes to adhere to osteopontin following PNS or CNS injury and oligodendrocyte proliferation, αβ3 correlates with astrocyte adhesion and oligodendrocyte differentiation and blockage of migration, and αβ8 promotes astrocyte migration (Fernandez-Valle et al., 1994; Milner et al., 1996; 1997; Stewart et al., 1997; Milner et al., 1999; Blaschuk et al., 2000; Dubovy et al., 2001; Colognato et al., 2004; Colognato & Tzvetanova, 2011). Integrin function from neuronal and glial cells as an integrated system will be discussed more in the following sections of this review.

2.3. Extracellular matrix proteins

ECM proteins are synthesized and secreted in the brain by neurons and glial cells. Neuronal ECM proteins include CN, FN, LN, proteoglycans, hyaluronan, reelin, and tenascins. The ECM receptors include integrins, dystroglycans, syndecans, and glypicans. This review is focus only on integrins, the major cell surface receptors for ECM. Other ECM receptor targets are beyond the scope of this review. In double-immunolabeling, reelin and the α3-integrin subunit are colocalized in a dendrite and a dendritic postsynaptic spine of adult nonhuman primate cortex (Rodriguez et al., 2000). Colocalization between α6 and LN, and α5 and FN have also been reported in embryonic neural crest cells (Strachan & Condic, 2004). ECM resembles a mesh-like structure surrounding cell bodies, proximal dendrites and axon initial segments and plays physical roles by serving as a structural support element or adhesive substrate. In addition, ECM molecules are present in basal lamina in the capillary and postcapillary venules, and constitute an essential part of the blood–brain barrier (BBB) (Ballabh et al., 2004). ECM also provides a signaling environment by serving as substrates for cellular receptors, thus triggering or influencing signaling events across cell membranes and providing a niche and anchor for signaling factors, and therefore regulates the bioavailability of signals. Neuronal cells do not only respond to the matrix through integrins, but they actively contribute to ECM formation and reorganization.

Cells determine the composition and quality of ECM and matrix receptors through differential gene expression, glycosylation, and

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Table 1

Integrin subunits and their endogenous ligands in the nervous and cardiovascular systems. Data compiled from: Glukhova and Koteliansky (1995); Pinkstaff et al. (1999); Velling et al. (1999); Chun et al. (2001); Moisera (2001); Ross and Borg (2001); Chan et al. (2003); Colognato et al. (2003); Morini and Becchetti (2010); and Wu et al. (2010).

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* In post-synaptics (muscle).
excluding FN and CN, contain the arginine might have different functions. Many neuronal matrix proteins, in-particular changes, or regulating the passage of growing or migratory processes of neuronal cell migration, axonal growth and myelination, proteins have pivotal functions at the BBB and contribute to the processes needed to modulate growth cone structure, neurite outgrowth and axon migration. This signal transduction has been also shown to result in protein phosphorylation, increased intracellular calcium concentration, activation of glutamate receptor, gene transcription, and the release of neurotransmitters (Clark & Brugge, 1995).

Intracellular signaling molecules (e.g. CSK and protein kinases) converge on the cytoplasmic domain of integrin tails after initial activation by ECM ligands can be described as three main activation states of rest, intermediate affinity state and activated high-affinity state (Cox et al., 2010), or 3 steps as inactive, active and clustering (Clark & Brugge, 1995). Integrins are not constitutively active. In general, integrins on oncogenic variants could be constitutively activated. Some integrins can bind their ligands in a resting state and then be activated, whereas others require activation before binding. Other than ECM ligands, extracellular factors such as mechanical stress (e.g. blood pressure), divalent cation concentration (e.g. Mn2+), and inter-leukin signaling also lead to integrin activation. After activation, integrin clustering by multivalent ECM proteins induces recruitments of CSK proteins (ECM–integrin–CSK axis) including actin, vinculin, talin, paxillin and tensin as well as NRTK kinases such as fak adhesion kinase (FAK) and the Src kinase family to the focal contact point forming into large multi-protein aggregates, termed cell-matrix focal adhesion complex (FAC). Mitogen-activated protein kinases (MEK), PLC-γ, and small GTPases rho, rac, and cdc42 as well as adaptor proteins such as Grb2 and Sos are also recruited to the ECM–integrin binding site after integrin activation (Fig. 1). Ligand binding induces conformational changes in integrins and intracellular signaling, referred to as “outside-in” signaling. This intracellular signaling cascades activated by integrin activation can result in the cytoskeletal changes needed to modulate growth cone structure, neurite outgrowth and axon migration. This signal transduction has been also shown to result in protein phosphorylation, increased intracellular calcium concentration, activation of glutamate receptor, gene transcription, and the release of neurotransmitters (Clark & Brugge, 1995).

Intracellular signaling molecules (e.g. CSK and protein kinases) converge on the cytoplasmic domain of integrin tails after initial ligand occupancy. In turn, ion channel activation, CSK remodeling, and intracellular signaling activities in neuronal cells can control integrin expression, activate the high affinity state of integrin (clustering), and manipulate remodeling of the ECM and ECM binding affinity onto integrin in the plasma membrane. Therefore, populations of integrins are not static, but change dynamically across periods of activation in response to physiological and pathological events. These alterations are referred to as “inside–out” signaling through the ECM–integrin–FAC axis (Clark & Brugge, 1995; Wu et al., 2001; Gall et al., 2006; Dityatev et al., 2007).

Different integrins may have distinct functions according to their ligand binding and type of intracellular signaling that is activated. Integrins can function as signaling receptors that transduce biochemical signals both into and out of cells (Clark & Brugge, 1995). Integrin activation by ECM ligands can be described as three main activation states of rest, intermediate affinity state and activated high-affinity state (Cox et al., 2010), or 3 steps as inactive, active and clustering (Clark & Brugge, 1995). Integrins are not constitutively active. In general, integrins on oncogenic variants could be constitutively activated. Some integrins can bind their ligands in a resting state and then be activated, whereas others require activation before binding. Other than ECM ligands, extracellular factors such as mechanical stress (e.g. blood pressure), divalent cation concentration (e.g. Mn2+), and inter-leukin signaling also lead to integrin activation. After activation, integrin clustering by multivalent ECM proteins induces recruitments of CSK proteins (ECM–integrin–CSK axis) including actin, vinculin, talin, paxillin and tensin as well as NRTK kinases such as fak adhesion kinase (FAK) and the Src kinase family to the focal contact point forming into large multi-protein aggregates, termed cell-matrix focal adhesion complex (FAC). Mitogen-activated protein kinases (MEK), PLC-γ, and small GTPases rho, rac, and cdc42 as well as adaptor proteins such as Grb2 and Sos are also recruited to the ECM–integrin binding site after integrin activation (Fig. 1). Ligand binding induces conformational changes in integrins and intracellular signaling, referred to as “outside-in” signaling. This intracellular signaling cascades activated by integrin activation can result in the cytoskeletal changes needed to modulate growth cone structure, neurite outgrowth and axon migration. This signal transduction has been also shown to result in protein phosphorylation, increased intracellular calcium concentration, activation of glutamate receptor, gene transcription, and the release of neurotransmitters (Clark & Brugge, 1995).

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et al., 2003; Morini & Becchetti, 2010). The inside-out signaling also occurs in regulating synaptic structure, signaling, and various forms of neuronal plasticity. In the brain, integrins are concentrated at sites of synaptic contact and are critical for the formation, maturation, and maintenance of synaptic structure (Nishimura et al., 1998). Basal synaptic communication requires that the presynaptic and postsynaptic faces of the synapse communicate via adhesive and intracellular signaling events (Schachner, 1997; Hoffman et al., 1998a).

Cleavage of integrin–ECM focal adhesion connections could be an early step in the formation of new synaptic configurations from inside-out signaling, followed by rapid formation of new focal contacts (Hoffman et al., 1998a).

2.5. Integrin crosstalk with cell surface receptors

As one of the cell surface receptors, integrins mediate crosstalk with other cell surface receptors such as growth factor receptors and cell adhesion molecules (CAMs) (Fig. 1). It is highly established that nerve growth factor (NGF) stimulates integrin-dependent axon outgrowth, but the mechanism connecting the two systems is not well understood. Integrins and growth factor receptors may activate parallel intracellular signaling pathways including extracellular signal regulated protein kinases (ERK)-type MEK. Co-stimulation of integrins and epidermal growth factor (EGF) receptors activates Pyk2 and FAK to promote outgrowth of neurite via paxillin induced cytoskeletal changes (Ivanovkovic-Dikic et al., 2000). Integrin clustering transactivates several receptor tyrosine kinases (RTKs) including platelet derived growth factor receptor (PDGF) and EGF receptor. Thus, integrins may increase signals generated by growth factor receptors through its FAC.

Transforming growth factor beta-1 (TGF-β1) latency-associated peptide is a ligand for the integrin αvβ6, and αvβ6 integrin expressing cells induce spatially restricted activation of TGF-β1. Mice lacking αvβ6 integrin develop exaggerated inflammation (Munger et al., 1999). Interactions between αv-integrin and growth factor receptor enable newly formed oligodendrocytes to survive in response to limiting concentrations of soluble growth factors (Colognato et al., 2002). The αvβ3 has been shown to promote the activation of TGFβ1 through binding to an RGD sequence in the TGFβ1 latency-associated peptide (Mu et al., 2002). Baron et al. have demonstrated a physical association between PDGF and αvβ3 receptors on oligodendrocytes. PDGF stimulated a protein kinase C-dependent activation of αvβ3 integrin, which in turn induced oligodendrocyte proliferation via a phosphatidylinositol-3-kinase-dependent signaling pathway (Baron et al., 2002).

It has been reported that synaptic remodeling would require alterations to interaction between integrin and cell adhesion molecules. Integrin mediate cell–cell interaction through cell adhesion molecules such as neural cell adhesion molecule (NCAM), intercellular adhesion molecule (ICAM), and vascular-cell adhesion molecule (VCAM). The NCAM L1 functionally interacts with β1-integrins to potentiate neuronal migration toward ECM proteins such as FN through endocytosis and MEK signaling, and that impairment of this function by L1 cytoplasmic domain mutations may contribute to neurological deficits in the X-linked mental retardation syndrome CRASH (corpus callosum agenesis, retardation, aphasia, spasticity, and hydrocephalus) (Thelen et al., 2002). During neuronal injury and cytokine stimulation, microglia bind to their targets using αIL-2 and α4β1 through interaction with ICAM-1 and VCAM-1 and migrating to the site through αMβ2 (Clegg et al., 2003).

Cross-talk has also been documented between integrin and cadherins. N-Cadherins and integrins function in a coordinated manner to effectively mediate the cellular interactions essential for adhesion and development. A peptide resembling the juxtamembrane (JMP) region of the cytoplasmic domain of N-cadherin results in release of the NRKT Fer from the cadherin complex and its accumulation in the integrin-induced FAC, and results in inhibition of N-cadherin and β1-integrin function. A peptide that mimics the first coiled-coil domain of Fer prevents Fer accumulation in the integrin cytoplasmic domain and reverses the inhibitory effect of JMP (Arregui et al., 2000). These results show that integrin activation is necessary to influence the function of other cell surface receptors and vice versa.

2.6. Integrin crosstalk with neurotransmitter receptors

Integrin could also have crosstalk with neurotransmitter receptors. It has been reported that α7β1 integrin colocalizes and physically interacts with LN-induced acetylcholine receptors (AChRs) clusters. LN through α7β1 facilitates the clustering of AChRs by a proteoglycan agrin in the post-synaptic membrane of skeletal muscle. Blocking antibodies to α7-, α7-, or β1-integrins inhibit the AChR clustering activity of LN and agrin (Martin & Sanes, 1997; Burkin et al., 1998, 2000). RGD-containing peptides or antibodies to the β3-integrin prevent a developmental increase in glutamate release and a concurrent shift in expression of postsynaptic N-methyl-D-aspartic acid (NMDA)-receptor subunits (Chavis & Westbrook, 2001). In rat hippocampal slices and synaptoneurosomes, a RGD peptide GRGDSP leads to potentiation of NMDA-gated currents, and increases the slope and amplitude of the fast excitatory postsynaptic potentials (EPSPs) because of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor activation (Lin et al., 2003; Bernard-Trifilo et al., 2005).

Integrin ligands such as RGD peptides and FN via β1-integrin increase EPSP in the hippocampus and could pass through two pathways. One of these pathways is through a Src kinase dependent NMDA and/or AMPA glutamate receptor pathway. Src tyrosine kinase increases protein phosphorylation of NMDA receptor subunits NR2A and NR2B in synaptoneurosomes and acute hippocampal slices. Another pathway is through a Src tyrosine kinase independent pathway. This pathway is related to activation of NMDA receptor, phosphorylation of Thr286 site on calmodulin-dependent kinase II, and phosphorylation of Ser831 site on AMPA receptors. The β1 integrin subunit or a mixture of function-blocking antibodies to α3, α5, and αv-integrin subunits block integrin ligand effects on synaptic responses. The γ-aminobutyric acid-A (GABAA) receptor antagonist picrotoxin does not reduce the enhanced effects of EPSP by integrin ligands (Kramer et al., 2003; Lin et al., 2003; Bernard-Trifilo et al., 2005). In β1-integrin knockout (KO) mice, expressions of AMPA and NMDA receptor subunits are normal. However, EPSPs at CA3–CA1 synapses are dramatically reduced, probably because of postsynaptic defect in AMPA receptor (Chan et al., 2008). These results show that hippocampal integrin activation is necessary to control the functional level of postsynaptic NMDA and AMPA receptors.

2.7. Integrin and Ca2+ signaling

Integrins modulate calcium signaling. The regulation of Ca2+ channels by integrin–ECM interactions may play a role in the regulation of neuronal migration, neurite extension and synaptic remodeling. Growth cone extension requires a permissive range of intracellular Ca2+ concentration, whereas the frequency of Ca2+ waves and spikes controls the rate of axonal elongation/spreading (Becchetti & Arcangeli, 2010). Neuronal Ca2+ channels in fresh isolated neuronal cells and heterologously expressed neuronal Ca2+ channel isoforms in HEK-293 cells are potentiated by α5β1 integrin. This potentiation is regulated by an intracellular signaling pathway involving phosphorylation of calcium channel subunit α1C, C-terminal residues Ser1001 and Tyr1122 by using truncation and site-directed mutagenesis strategies (Gui et al., 2006; Wu et al., 2010a). These sites are known to be phosphorylated by PKA and C-Src, respectively. Soluble RGD containing peptide ligands increase the expression of mRNAs for the neurotrophin brain derived neurotrophic factor, nerve growth factor, neurotrophin-3, the neurotrophin receptors TrkB
and TrkC, and c-fos at least in part through effects on calcium influx in adult hippocampus (Gall et al., 2003). In this context, integrin-αC-β1 channel interactions appear to be critical for the up-regulation of brain-derived neurotrophic factor mRNA in hippocampal neurons. Work in cerebellar neurons, immune cells, and epithelial keratinocytes suggests that a stable rear-to-front \( \text{Ca}^{2+} \) gradient is formed in cells during chemotaxis/migration, which is thought to be one of the causes of the different behavior of the front and rear sides, with retraction and detachment of the trailing edge during cell movement cycle.

Integrin-αC-β1 channel interactions could also play a major role in the responses of neurons and blood vessels to injury and repair (e.g. stroke). A series of observations from our previous studies show that at least 3 different integrins regulate Ca\( _2^{2+} \) activated K+ channels and myogenic responses in vascular systems, and in heterologously expressed smooth muscle Ca\( _2^{2+} \) in HEK cells through inside-out and outside-in signaling between integrin and FAC (Wu et al., 1998, 2001; Davis et al., 2002; Waitkus-Edwards et al., 2002; Gui et al., 2006; Wu et al., 2008b; Yang et al., 2010). Both neuronal activity and vascular reactivity are modulated by integrin signaling through the generation or exposure of new integrin ligands from limited degradation of ECM and/or turnover of new integrins during wound healing (Davis et al., 2000; Gui et al., 2006). For instance, successful axonal regeneration is highly correlated with the induction of integrins on the surface of peripheral neurons; therefore, peptides derived from integrin ligand have the potential to act as therapeutic agents for neuronal regeneration (Meiners & Mercado, 2003; Cox et al., 2010).

2.8. Integrin in nervous system development

Integrins play a major role in the development of the CNS by stabilizing cellular contacts during cell migration, cell proliferation, and neurite extension. Integrin and FAC signaling molecule recruitment (e.g. GTPases Cdc42, Rac1 and CSK actin at leading edge), and integrin-ECM detachment/deadhesion (at trailing edge) and efficient attachment/adhesion have been reported to dynamically change their position at leading and trailing edges in migrating cells during development (Becchetti & Arcangelii, 2010; Huttenlocher & Horwitz, 2011). Lacking ECM components such as LN or FN, or lacking the β1-family of integrins, mice die during the early steps of embryonic development. The loss of α4, α5, or/and β1-integrins from the mice embryo results in abnormal lamination of the cortex and cerebellum due to disruptions of the basal lamina that separates the brain from the overlying mesenchyme, and results in severe perturbations of the peripheral nervous system, including failure of normal nerve arborization, delay in Schwann cell migration, survival, proliferation, and differentiation, and defective neuromuscular junction differentiation (Graus-Porta et al., 2001; Haack & Hynes, 2001; Feltri et al., 2002; Pietri et al., 2004). These alterations are likely to reflect the roles of integrin receptors in regulating activation of MEK, Rac, and other signaling pathways (Campos et al., 2004).

In an in vitro injury assays with function-integrin antibodies in avian neural crest cells, cell spreading is essentially mediated by FN receptor αvβ1 and αvβ8, and migration involved αvβ3, αvβ5 and αvβ8 (Testaz et al., 1999). In LN receptor α6-integrin KO mice, the cortical basement membrane is disrupted with alterations of LN deposition, ectopic neuroblastic outgrowths are found on the brain surface and in the vitreous body in the eye, and myelin-forming oligodendrocytes show increased cell death (Georges-Labouesse et al., 1998). α3-integrin KO mice show a disorganized cortex and defective neuronal migration (Anton et al., 1999). The α5β1 integrin as FN receptor has been implicated in regulating the morphology of cortical development and dendritic spines, formation of synapses in neurons, as well as in mediating neurite outgrowth after injury. Embryos lacking the α5-integrin subunit die at embryonic day 10.5 before cerebral cortex development (Marchetti et al., 2010). No obvious phenotypes in the peripheral nervous system have been reported in mice lacking the αv- or β8-integrins. However, expression of αvβ8 is required for normal vascular development in the CNS. Absence of either αv or β8 integrins in the CNS leads to cerebral hemorrhage, seizures, axonal degeneration and premature death (McCarty et al., 2005, Proctor et al., 2005).

In the future, conditional mutation on more subtle genetic models for integrins, ECM proteins, and FAC molecules will allow a more detailed understanding on the highly complicated function of integrin-mediated adhesion in the development of nervous system.

3. Physiological and pathological roles of integrins in brain behavior and brain disorders

Very little is known regarding the role of integrins in neurophysiological and pathological conditions. Emerging evidence shows that integrin and ECM production are altered in physiological and pathological conditions such as memory, tumor, Alzheimer’s disease, stroke, and epilepsy (Clegg et al., 2003; Gall & Lynch, 2004; Dityatev & Fellin, 2008). Although integrins are known to be involved in various physiological and brain disorders, identifying the specific integrin involved and their precise role are difficult because many diseases are multifactorial and integrins are only one of these receptors involved. In this section, we will discuss recent reports on integrin role in the pathophysiology of learning and memory, and selected brain disorders.

3.1. Learning and memory

Integrins are involved in learning and memory through modulation of long-term potentiation (LTP). The LTP has long been regarded as a plausible cellular substrate for learning and memory. At least two essential biochemical phases exist for LTP linked learning and memory: an early phase involving modification of existing synaptic proteins, and a late phase conveyed by de novo protein synthesis and formation of new synapses. It is thought that integrins help to form synapses in learning and memory. Integrins are involved in activity-dependent synaptic plasticity and in spatial memory. Evidence for a potential role in synaptic plasticity has been gathered by attenuating the stability of hippocampal LTP by using broad-spectrum peptide inhibitors of integrins or other pharmacological reagents. A direct link of integrin function to memory formation is demonstrated in Drosophila, in which the disruption of Volado, a gene encoding for two forms of the α-integrin, impairs short-term olfactory learning. Conditional expression of an integrin subunit rescued the memory deficits (Groteviel et al., 1998).

Furthermore, disruption of the integrin-associated protein produces memory deficits in mice (Huang et al., 1998; Chang et al., 1999, 2001). Two vertebrate integrins of α8 and β8 have been localized to dendritic spines of pyramidal neurons where they are associated with postsynaptic density (Einheber et al., 1996; Nishimura et al., 1998). The α5 integrin has been shown to distribute preferentially to apical dendrites of pyramidal cells of the hippocampus and neocortex (Bi et al., 2001). Four different integrins of Drosophila have been localized to the presynaptic and/or postsynaptic side of the larval neuromuscular junction (Prokop, 1999). Heterozygous mutants of the integrin gene α3-subunit, but not of α5- or α8-subunit, reduce the magnitude of NMDA receptor dependent hippocampal LTP. However, when the expression of the three integrin genes, α3-, α5- and α8-subunits, is reduced simultaneously, a deficiency in spatial memory is produced but fear conditioning remains normal. These functional changes are associated with a fairly rapid but subtle morphological change in the structure of the synapse such that synaptic transmission is altered (Chan et al., 2003).

Electrophysiological studies demonstrated that mutation or removal of β1-integrin have impaired synaptic transmission through AMPA receptors and diminished NMDA receptor-dependent LTP, and impaired in some kind of memories (Chan et al., 2006; Huang et al.,...
In addition, the \( \beta_1 \)-integrin receptors and their ECM ligands (FN, LN, and CN) have been implicated in the process of neurite outgrowth and LTP (Venable & Reichardt, 1993; Chan et al., 2003; Huang et al., 2006). Neurite outgrowth is a process commonly thought to contribute to LTP by formation of new synaptic contacts that can be detected in the process of learning and memory. Activation of \( \beta_1 \)-integrin or several \( \beta_1 \)-binding \( \alpha \)-integrins, including \( \alpha_3 \), \( \alpha_5 \), and \( \alpha_8 \), further initiate intracellular signaling and transcription and translation, and facilitate LTP related learning and memory.

Integrin ligands participate in the process of learning and memory. ECM molecule tenasin-C and a fragment of tenasin-C containing the fibronectin type-III repeats 6–8 are involved in hippocampus-dependent contextual memory and synaptic plasticity (Strekalova et al., 2002). The disruption of integrin–ECM interactions using RGD peptide and other integrin antagonists also interferes with the maintenance of use-dependent synaptic reorganization and LTP. Hippocampal synapses are enriched in FN receptors (i.e. \( \alpha_5 \beta_1 \) integrin) that contribute importantly to the stabilization of LTP (Bahr et al., 1997; Staubli et al., 1998; Rohrbough et al., 2000; Chun et al., 2001; Kramar et al., 2002). Overall, integrins are essential for neuroplasticity in LTP and memory. Thus, future studies that elucidate the signaling cascade triggered by integrin engagement at the synapses will provide new insight into the processes of learning and memory.

3.2. Aging, Alzheimer’s disease and Down’s syndrome

Integrins may play roles in aging and age-related neurological disorders. Integrins are involved in synaptic plasticity in neurodegenerative conditions and immune response to the diseases. In humans, hippocampal pyramidal neurons and some neocortical neurons showed immunoreactivity with \( \alpha_4 \)-integrin subunit and FN in all aged individuals, but not in younger patients. Antibodies against the \( \alpha_4 \)-integrin subunit and a FN specific antibody also stained the tau-positive plaques (i.e. neuritic-type plaques) in Alzheimer’s disease and Down’s syndrome, while ‘preamyloid’ plaques remained negative (Van Gool et al., 1994). In addition, many senile plaques and neurofibrillary tangles in Alzheimer brain tissue are prominently stained for high level VN and VN receptor \( \beta_3 \)-integrin (Akiyama et al., 1991). The \( \alpha_4 \beta_1 \) and \( \alpha_6 \beta_1 \) integrins in activated microglial cells adjacent to amyloid plaques indicate inflammatory responses in Alzheimer disease (Priciato-Patt et al., 1996). In rat hippocampal neurons and rat cortical astrocytes, cell surface \( \beta_5 \)-amyloid precursor protein is colocalized selectively with \( \alpha_1 \beta_1 \) and \( \alpha_5 \beta_1 \) integrins. In transfected human neuroblastoma cell line, \( \alpha_5 \beta_1 \) integrin appears to mediate the internalization and degradation of exogenous \( \beta_5 \)-amyloid. When deposition of insoluble amyloid around the \( \alpha_5 \beta_1 \)-expressing cells is reduced, the cells show less apoptosis than the control cells (Yamazaki et al., 1997; Matter et al., 1998). Further studies on integrin such as \( \alpha_4 \beta_1 \) and \( \alpha_5 \beta_1 \) integrins in human brain tissue may provide more insight on the role of integrins in aging and Alzheimer’s disease.

3.3. Injury and stroke

Integrins are believed to play an important role in neuronal injury and ischemic stroke. FN and \( \alpha_5 \beta_1 \) integrin are expressed at comparatively high levels in developing nerve, and less prominently expressed during nerve maturation. Following lesion of mature nerve or hippocampal hilus lesion by surgery, the up-regulated expression of FN and \( \beta_1 \)-integrin (e.g. \( \alpha_5 \beta_1 \) integrin) are in the vicinity of the lesion, in the growth cones of regenerating neurons and on Schwann cells (Lefcort et al., 1992; Pinkstaff et al., 1998). During peripheral neural repair and regeneration, activated integrins in microglia promotes adhesion, endocytosis, phagocytosis, and break down of cellular debris. Increased \( \beta_1 \)-integrin in neuronal cells relates to neuronal adhesion and neurite outreach and regeneration (Clegg et al., 2003). Mature CNS neurons are believed to lack the capacity to regeneration after injury except dentate granular cells (DGs) in the hippocampus. However, the regenerative performance of adult neurons can be restored to that of young neurons by gene transfer-mediated expression of a single \( \alpha \)-integrin. Transfection of adult neuronal cells with \( \alpha_1 \)-integrin results in increased neurite outgrowth on FN, while transfection with \( \alpha_5 \)-integrin enhances neurite outgrowth on FN in vitro (Condic, 2001). Genetic variants with integrin \( \alpha_2 \)-subunit have been reported to be associated with an increased risk for ischemic stroke (Matarin et al., 2008). After focal ischemic stroke, increased synthesis and release of matrix proteins osteopontin to the normal brain, and the increased expression of integrin \( \alpha_4 \beta_3 \) indicated that osteopontin plays a novel role in glial activation, organization, and repair functions (Ellison et al., 1999). Following stroke, \( \beta_1 \)-integrin is increased in cerebral blood vessel in ipsilateral ischemic cortex, and is involved in modulating angiogenesis in ischemic stroke (Lathia et al., 2010).

In both rat and mouse stroke models, western blot analysis revealed elevated levels of ECM fragment of perlecan (domain V). Post-stroke domain V administration increased vascular endothelial growth factor levels through brain endothelial cell \( \alpha_5 \beta_1 \) integrin, and the subsequent neuroprotective and angiogenic actions of perlecan domain V were in turn mediated through vascular endothelial growth factor receptors (Lee et al., 2011). Becker et al. reported that treatment with \( \alpha_4 \)-integrin antibody (Ab) decreased infarct size, increased lymphocyte/monocyte and lowered neurological deficit scores in rat focal cerebral ischemia model (Becker et al., 2001). These results suggest that integrins could represent a promising approach for stroke treatment.

3.4. Epilepsy

There is emerging evidence on the role of integrins in epilepsy. Epilepsy is the second most common neurological disorder that affects over 3 million people in the US and about 50 million worldwide (Browne & Holmes, 2001). It is a disorder in which the balance between neuronal excitability and inhibition is disrupted, leading toward uncontrolled synchronized excitability. In temporal lobe epilepsy (TLE), the hippocampus plays a key role in epileptogenesis and epileptic seizures (Schwartzkroin, 1994; Reddy, 2010). Ablant expressions of neurotransmitter receptors, cytoskeletal proteins, synaptic proteins, antioxidant proteins, and MEK have been found in the hippocampus of patients with TLE (Furtinger et al., 2003; Notenboom et al., 2006; Yang et al., 2006; Perosa et al., 2007). In addition to these intracellular modifications, there is increasing evidence for a functional correlation between the localization of integrins in the hippocampus and the role that integrins may play in neuronal epileptiform activities (Chang et al., 1993; Grooms & Jones, 1997).

Integrins are differently distributed in cellular and subcellular compartments in the hippocampus regions and undergo specific patterns of regulation during development of epilepsy. Immunochemical and co-precipitation studies have suggested 11 potential integrin receptors with species difference in the hippocampus including \( \alpha_1 \beta_1 \), \( \alpha_2 \beta_1 \), \( \alpha_3 \beta_1 \), \( \alpha_4 \beta_1 \), \( \alpha_5 \beta_1 \), \( \alpha_6 \beta_1 \), \( \alpha_8 \beta_1 \), \( \alpha_5 \beta_3 \), \( \alpha_5 \beta_5 \) and \( \alpha_5 \gamma_8 \) (Fig. 2) (Pinkstaff et al., 1999; Chun et al., 2001; Chan et al., 2003; Fasen et al., 2003). Significant hippocampal immunoreactivity of \( \alpha_1 \), \( \alpha_5 \), \( \beta_1 \), \( \beta_3 \), \( \beta_4 \), and \( \beta_5 \)-integrins is observed in the pla mater, in vascular endothelia, and in astrocytes at the pial surface; immunoreactivity of \( \beta_2 \)- integrin is found exclusively in vascular endothelia, and immunoreactivity of \( \alpha_2 \), \( \beta_4 \), and \( \beta_5 \)-integrins is found in mossy fibers. Pyramidal cells, internuclei of CA1–CA3, and hilar neurons reveal moderate \( \alpha_5 \)-integrin labeling in their cell bodies. After pilocarpine-induced status epilepticus, strong immunoreactivity for \( \alpha_1 \), \( \alpha_2 \), \( \alpha_4 \), \( \alpha_5 \), \( \beta_1 \), \( \beta_3 \), and \( \beta_4 \)-integrins is observed in reactive astrocytes (Fasen et al., 2003). Seizures induce marked increases in \( \alpha \)- and \( \alpha \)-integrins that are restricted to neuronal cell layers and are particularly striking in hippocampal stratum granulosum, CA1 and CA3.
Our immunocytochemistry data show at least 20% increase in α5-integrin expression in hippocampal DGCS in stage 5 kindling mice (unpublished data). In contrast, seizure-induced increases in α6-integrin are more diffusely distributed and are localized in both neurons and glia (Gall & Lynch, 2004). The β1-integrin expression is reported broadly increased and have been implicated in the process of neurite outgrowth during the seizure episode (Venstrom & Reichardt, 1993; Gall & Lynch, 2004). Hippocampal neurons and astroglial cells express quite low levels of β1-integrin mRNA in naïve rats. However, β1-integrin expression can be dramatically increased in response to seizure within hippocampal stratum pyramidale and the dentate gyrus hilus. β1-integrin mRNA levels increased first in neurons and peaked with predominant astroglial expression at 24 h after seizure (Pinkstaff et al., 1998). Laminin β1-subunit and integrin α2-subunit expression are also elevated in the anterior temporal neocortex tissue from patients with intractable epilepsy (Wu et al., 2011b).

Integrins as unique candidates may participate in epileptogenesis. There is increasing evidence for a functional correlation between the localization of integrins in the hippocampus and the role that integrins may play in neuronal epileptiform activities (Chang et al., 1993; Grooms & Jones, 1997). Seizure activity has been shown to induce a ‘matrix response’. These responses included release of protease, changes in the localization and synthesis of ECM molecules (e.g., FN, reelin, and glycosaminoglycans), and activation of integrin–intracellular signaling pathways following seizure activity in the hippocampus (Hoffman et al., 1998b, 1998c; Nafah-Mazzocarotti et al., 1999; Perosa et al., 2002a, 2002b; Gong et al., 2007). In animal epilepsy and in human TLE, neurons in hippocampus undergo extensive remodeling, including acute neurodegeneration and intracellular signaling adjustments, and chronic processes including reorganization of mossy fibers, dispersion of the DGC layer and the appearance in ectopic locations within the dentate gyrus. Integrin recruitment and relocalization has been observed in migrating cells, and integrin–ECM attachments and focal adhesions have been reported to dynamically change their position at leading and rear edges (Becchetti & Arcangeli, 2010; Huttenlocher & Horwitz, 2011). Our data show decreased FN–integrin binding force/adhesion force and increase cell membrane stiffness/plasticity in DGCs from stage 5 kindling mice by using nanoscale atomic force microscopy (unpublished data). These results indicate alternation of ECM–integrin interaction represented as ECM–integrin binding changes and alternation of intracellular CSK remodeling and intracellular molecule activation represented as stiffness/plasticity changes during epilepsy.

As we mentioned above, epilepsy is a disorder in which the balance between neuronal excitability (e.g., NMDA currents) and inhibition (e.g., GABA currents) is leaned toward uncontrolled excitability. Integrin expression is greatest in glutamatergic (NMDA) neurons and low in GABAergic neurons and glia, and concentrated in specific areas such as synaptic membrane (Gall & Lynch, 2004). There are several reports regarding β1-integrin family activated excitatory NMDA currents and Ca2+ currents in neurons as discussed above (Lin et al., 2003; Bernard-Trifilo et al., 2005; Lin et al., 2005; Gui et al., 2006; Lin et al., 2008). All of these changes will increase glutamate excitability and burst activities in hippocampal CA1 and DGC, cause hyperexcitation in neurons, and could be primary events leading to development of the epilepsy (Tauck & Nadler, 1985; Sutula et al., 1985; de Lanerolle et al., 1989; Chang et al., 1993; Wuaarin & Dukde, 2001; Smith & Dukde, 2002; Lin et al., 2003; Bernard-Trifilo et al., 2005; Lin et al., 2005; Gui et al., 2006; Lin et al., 2008). In epileptic conditions, increased neuronal proliferation in the DGCs which are principal excitatory glutamatergic phenotype neurons, cause hyperexcitation (Kokai et al., 2011). However, there are still limited reports available on whether or how integrin directly or indirectly modulates GABA channels. Colocalization of α3-integrin subunit and the GABA receptor β2/3 subunit has been reported in the Purkinje neuron. Activating α3/1 integrin impairs the GABAergic miniature inhibitory postsynaptic currents (Kawaguchi & Hirano, 2006). ECM protein tenascin-R–deficient mutant mice show impaired LTP, elevated levels of excitatory synaptic transmission, and reduced levels of perisomatic inhibitory currents mediated by GABA_α receptors in CA1 of the hippocampus (Bukalo et al., 2007). These reports reveal the complex interplay between integrins and GABAergic neurotransmitter receptors. Because glutamatergic activities are related to LTP as mentioned above, these results indicate that integrin contributions to plasticity are not restricted to ‘good things’ as LTP to memory, but included ‘bad things’ as long lasting increases in excitatory LTP, strengthen the network and connectivity, and lower the threshold for epileptogenesis.

Epileptogenesis involves a local breakdown of normal cell–cell and cell–matrix relationships followed by new synthesis of ECM and integrins. During normal neural development, ECM molecules play an important role in neuroplasticity events such as neurogenesis, axonal outgrowth, and synaptogenesis. Synaptogenesis is associated with the early stages of epilepsy formation and these plasticity changes are also involved in the development of epilepsy (Chang et al., 1993). ECM–integrin interactions may alter neuronal signaling through the RGD binding site or increasing the number of activated integrins in epilepsy (Grooms & Jones, 1997; Morini & Becchetti, 2010). The RGD-treated brain slices display a significant decrease in the spontaneous burst rate (epileptic-like activities), but the period of spontaneous bursting increase dramatically in the hippocampus CA3. This paradoxical effect might be because RGD peptide decreases the number of available integrin-RGD containing ECM protein binding sites, which may interfere with neuronal communications required for synchronized electrical firing rates (Grooms & Jones, 1997). Kainic acid-induced seizure caused a broadly distributed increase of FN as early as 1 h after recurrent seizure in adult brain hippocampus including dentate gyrus hilus and cortex as well as a larger focal increase in the ventral subiculum. FN density is higher in cell hillock within individual cells in dentate gyrus hilus (Hoffman et al., 1998c). The hippocampus of TLE patients show increased levels of chondroitin sulfate and hyaluronic acid, another two components of ECM protein glycosaminoglycans. The increased concentration of hyaluronic acid and chondroitin sulfate in the hippocampus of TLE patients demonstrates the importance of the matrix compounds during neosynaptogenesis, neurite outgrowth, and the mossy fiber sprouting found in temporal lobe epilepsy phenomena (Perosa et al., 2002a).

The expression of ECM molecule tenascin-C, which could bind to α6β1, α9β1, αvβ3 and αvβ6 in nervous system (Varnum-Finney et al., 1985; Nakic et al., 1996; Shapiro et al., 1996; Clegg et al., 2003), reaches peak upregulation at 24 h after kainic acid-induced limbic seizures in the CA1 and granule cell layer of adult rat, coincident with activation of granule cells and sprouting of axon terminals. The remaining tenasin expression 30 days after injection only relates to pyramidal cells in CA1 and CA3, coincident with an astroglial response (Nakic et al., 1996). During status epilepticus, overall ECM protein hevin SC1 levels decrease in DGC. A transient increase in SC1 co-localization with the cellular stress marker Hsp70, the degeneration marker Fluoro-Jade B, and the neuron activity marker activity-regulated CSK-associated protein are also shown in neurons of the hippocampal CA1, CA3, and hilar regions after status epilepticus. The levels of SC1 protein in neurons of the hippocampal seizure-resistant CA2 region do not change throughout the seizure (Lively & Brown, 2008).

α-Tubulin, a component of ECM–integrin–FAC (or CSK) axis, exhibits increased expression in CA3 and DGC bodies, dendrites and axons of hippocampus in epilepsy models. Microtubule formation may contribute to synaptic remodeling, such as mossy fiber sprouting and reorganization of neural networks of kindling-induced epileptogenesis (Pollard et al., 1994; Hendriksen et al., 2001; Sato & Abe, 2001). However, in mesial TLE patients, tubulin α-1 chain, β-tubulin,
4. Therapeutic implications of integrins as drug targets

Integrins are being explored for designing drugs for the treatment of neurological disorders. Because successful axonal regeneration of attachment, migration, and extension processes are highly correlated with the induction of integrins on the surface of neurons, peptides or other types of ligands derived from ECM proteins may have therapeutic potential for treatment of brain diseases (Meiners & Mercado, 2003).

4.1. Approaches to integrin drugs

Rapid progress has been made in the discovery and development of integrin targeted therapies in the past 15 years. The first integrin-specific drugs target the platelet integrin, αIIbβ3 and have been marketed for acute coronary syndromes and prevention of myocardial infarction following percutaneous coronary intervention since the late 1990s. This drug has been proven to be effective and safe and contributed to the reduction of mortality and morbidity in acute coronary syndromes. There are numerous in vitro, in vivo, preclinical and clinical studies implementing integrins as potential drug candidates for human diseases (Table 2). There are several mechanisms to modulate integrin function for drug development. The first mechanism as the original strategy for currently approved inhibitors is to block the ligand binding (i.e. block adhesion). Integrin inhibitors targeted on three integrins of αIIbβ3, α4 and αLβ2 have been approved. Currently, integrin Abs, integrin ligand peptides and small non-peptide inhibitors have been clinically targeted. Abs can have longer circulation lifetimes compared to small peptide-based and non-peptide drugs thus increasing the duration of therapy. However, the disadvantages associated with antibody therapeutics are the high cost of production, the need for intravenous administration, and the propensity for host immunogenicity and infusion reactions (Cox et al., 2010; Millard et al., 2011).

The second mechanism is to modulate integrin expression and activation. As recent advances in our understanding of ECM–integrin–intracellular signaling axis, blocking downstream integrin signaling for both outside-in and inside-out will modulate integrin expression and activation. For instance, CSK talin binding, known for forming the initial contacts between integrin β-subunit intracellular tails and the actin cytoskeleton, is a pivotal event and is required specifically for integrin–ECM–CSK axis signaling and for activation of integrin (Kim et al., 2011; Millard et al., 2011). If talin binding to intracellular β-integrin tail is inhibited, it will prevent inappropriate integrin activation and block subsequent integrin signaling. Similarly, NRTKs such as FAK and Src binding to β-tails and CSK protein paxillin binding to α-subunit intracellular tails represent additional potential key targets for development of selective inhibitors in ECM–integrin–FAC pathways. Some inhibitors such as HYD1 for αIIb3 and ATN-161 for FN sequence to block signaling have been studied in clinical or pre-clinical trials (Cianfrocca et al., 2006; Sroka et al., 2006).

4.2. Potential clinical applications

Integrin targeted agents may have clinical applications in brain disorders. Integrins regulate the transit of lymphocytes, macrophage, and other cells across the blood–brain barrier in response to inflammatory stimuli, anti-integrin reagents are of great interest as therapeutic agents to control demyelinating diseases, such as multiple sclerosis that involve the immune system and inflammatory responses. A new potential approach that complements traditional therapy, targeted to specific integrin receptors using integrin ligands, has been reported. The anti-α4-integrin Ab natalizumab, which inhibited attachment of immune-competent cells (leukocyte) to inflamed brain endothelium,

Table 2
Integrin as target of drugs. Data compiled from: Horwitz (1997); Clemetson and Clemetson (1998); Wu and Davis (1998); Ross and Borg (2001); Staunton et al. (2006); and Millard, et al. (2011).

<table>
<thead>
<tr>
<th>Integrin</th>
<th>Cell types</th>
<th>Integrin ligands</th>
<th>Example of disorder indication and drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>αIIbβ3</td>
<td>Platelets</td>
<td>Fibrinogen, von Willebrand factor, FN, VN</td>
<td>Acute coronary syndrome, MI, restenosis after angioplasty, percutaneous coronary intervention, thrombosis. Drugs: Abciximab, Eptifibatide, Abeximab, etc.</td>
</tr>
<tr>
<td>3/2 integrins (CD18)</td>
<td>Various white blood cells</td>
<td>ICAM</td>
<td>Reperfusion injury, stroke, shock, MI, multiple sclerosis, psoriasis, chronic inflammatory diseases, meningitis. Drugs: Erlizumab, Efalizumab (αIIb3)®, etc.</td>
</tr>
<tr>
<td>β1 integrins (CD29)</td>
<td>Widespread including nervous system</td>
<td>FN, LN, CN, others</td>
<td>Platelet aggregation, angiogenesis, homeostasis, wound healing processing, hypertrophy. Drugs: see individual αi-integrin.</td>
</tr>
<tr>
<td>α4β1(1) (CD49d)</td>
<td>Various white blood cells, nervous system</td>
<td>FN, VCAM</td>
<td>Multiple sclerosis, chronic inflammatory diseases, such as Crohn’s disease, asthma and arthritis. Drugs: Tysabri, Natalizumab.</td>
</tr>
<tr>
<td>α5β1 (CD49e)</td>
<td>Smooth muscle cells, cardiomyocytes, nervous system</td>
<td>FN (possible biomarker for infarction)</td>
<td>Restenosis, muscle dystrophy, tumors, age-related macular degeneration. Drugs: Volociximab, etc.</td>
</tr>
<tr>
<td>αvβ3 (CD51)</td>
<td>Endothelial cells, vascular smooth muscle</td>
<td>FN, VNAS</td>
<td>Angiogenesis, glioblastoma, diabetic retinopathy, heart defect, atherosclerosis, prostate cancer, pancreatic cancer, melanoma and postmenopausal osteoporosis. Drugs: Gleegotide, Vitasin, MK0429, etc.</td>
</tr>
<tr>
<td>α4β7</td>
<td>Various white blood cells</td>
<td>FN, MadCAM, VCAM</td>
<td>Chronic inflammatory diseases, such as ulcerative colitis. Drugs: MDM2</td>
</tr>
</tbody>
</table>

FN: fibronectin; VN: vitronectin; CN: collagen; LN: laminin; MadCAM: mucosal addressin cell adhesion molecule; VCAM: vascular cell adhesion molecule; ICAM: intercellular adhesion molecule; MI: myocardial infarction.

a FDA approved integrins that can be used in diseases are in bold font.

b Withdrawn from market in 2009.
demonstrated an unequivocal therapeutic effect in preventing relapses and slowing down the pace of neurological deterioration in patients with relapsing–remitting multiple sclerosis. Combinational treatment with both anti-\( \alpha \)-\( \beta \)-7 and \( \alpha \)-4 integrin subunit Abs led to more rapid and complete remission than that obtained with anti-\( \alpha \)-4 antibody alone. These results confirmed that \( \alpha \)-4\( \beta \)-1, \( \alpha \)-4\( \beta \)-7, and \( \alpha \)-2\( \beta \)-7 integrins may all play a contributory role in the progression of chronic forms of demyelinating disease, and together with their ligands could represent potential targets for improved treatment of some forms of multiple sclerosis (Bartt, 2006; Engelhardt & Kappos, 2008; Kanwar et al., 2000). Treatment with natalizumab (\( \alpha \)-4-integrin) also led to a dramatic reduction of refractory seizures in a patient with multiple sclerosis (Sotgiu et al., 2010). The involvement of integrins in inflammatory responses has also created interest in the possibility of using anti-integrin reagents to alleviate several neurodegenerative disorders, including Parkinson’s and Alzheimer’s diseases. Of special interest, \( \alpha \)1\( \beta \)-1, \( \alpha \)6\( \beta \)-1 integrins and CD47 (integrin-associated protein) appear to interact with amyloid precursor protein and these are postulated to mediate deposition or toxic actions of A and amyloid formation (Bozzo et al., 2004; Koenigsknecht & Landreth, 2004).

Integrin antagonists that target angiogenesis for brain tumors are progressing through clinical trials. Clengidite, a selective inhibitor of \( \alpha \)3\( \beta \)-3 and \( \alpha \)5\( \beta \)-3 integrins, inhibits cell signaling through focal adhesion kinase/Src/Akt and extracellular signal-regulated protein kinase mediated pathways, and attenuates the effect of vascular endothelial growth factor stimulation on growth factor signaling in endothelial and glioma tumor cells. Following clengidite treatment, endothelial and glioma cells show disassembly of cytoskeleton and disruption of tight junction formation (Oliveira-Ferrer et al., 2008). The addition of clengidite to chemoradiotherapy based treatment regimens has shown promising preliminary results in ongoing clinical trials in newly diagnosed and progressive glioblastoma multiforme patients by inhibiting angiogenesis (Millard et al., 2011). The greatest challenge facing the development of anti-angiogenic integrin targeted therapies is the overall lack of biomarkers by which to measure treatment efficacy.

4.3. Drug delivery in nervous system

The major problem in drug delivery to CNS is the presence of the blood–brain barrier (BBB) and blood cerebrospinal fluid barrier, mechanisms that protect CNS against intrusive chemicals but also confound therapeutic interventions. Compared to BBB, blood cerebrospinal fluid barrier allows the free-movement of small molecules. Many existing drugs including current clinically available integrin drugs are rendered ineffective in the treatment of many CNS diseases due to our inability to effectively safely deliver, and sustain them within the CNS. To circumvent the barriers, at least three strategies are practically possible and have been evaluated. There are manipulating drugs, disrupting the BBB and discovering alternative routes for drug delivery (Misra et al., 2003). Designer drugs are prepared through increasing the lipophilicity of therapeutic drugs, and vector (e.g. nanoparticles) or receptor and carrier (e.g. levodopa) mediated drug delivery. Disrupting the BBB is carried out through hypertonic solution initiating endothelial cell shrinkage and vasoactive leukotriene increasing cell permeability. These two strategies are delivered via the circulatory system and frequently result in unwanted side effects. The third strategy is to bypass the BBB and deliver the drugs to CNS through an intraventricular/intrathecal route, the olfactory pathway, an interstitial/intracranial drug delivery via releasing drugs from biological tissues, nanoparticles and pumps.

Nanoparticles as a drug-carrier system for the antiepileptic valproic acid have been studied in mice. This study suggested that nanoparticles loaded with valproic acid may help to reduce the toxic side effects of valproate therapy, not by reducing the therapeutically necessary dosage but by inhibition of formation of toxic metabolites (Darius et al., 2000). Wilson et al. (2008) have used poly-nanoparticles for the targeted delivery of rivastigmine into the rat brain to treat Alzheimer’s disease. Targeted gene therapy with \( \alpha \)3\( \beta \)-3 integrin-binding nanoparticles as treatment for choroidal neovascularization has also been reported (Salehi-Hash et al., 2011). Sniffling (intranasal) neuropeptides of melatonin, vasopressin and insulin achieved direct access to the cerebrospinal fluid within 30 min, bypassing the bloodstream in human trials (Born et al., 2002). RGD peptides are small peptides that could be applied through this pathway. In addition, application of multifunctional nanocarriers with integrin ligands across the BBB, allowing for diagnostic and therapeutic agents to be selectively targeted on a specific tissue and cell, also minimizing exposure to the healthy tissue and cell, is one of enormous challenges for biological discovery and clinical practice.

BBB dysfunction in the hippocampus could be present in a chronic hypertensive state at an early stage, in aged senescence-accelerated mice, and during inflammatory reaction. BBB structure could be compromised during stroke, tumor metastasis, and injury (Ueno et al., 2004; Pelegri et al., 2007; Cox et al., 2010). These could provide an opportunity for applying anti-integrin drugs to CNS.

The road from basic research to clinical application of integrin pharmacology has been very challenging. Approximately 2–4% of patients had anaphylaxis or other hypersensitivity reactions to natalizumab. Combined natalizumab and interferon-\( \alpha \)-1a therapy developed progressive multifocal leukoencephalopathy in multiple sclerosis because of their immune suppressive properties (Engelhardt & Kappos, 2008; Warnke et al., 2010). Thus Natalizumab and related \( \alpha \)-4-integrin targeting drugs come with a black-box warning on the drug label and are now limited to patients refractory to standard therapies. In clinical practice, physicians face the tough challenge of accurately evaluating both the benefits and the risks of therapy for the patients. The real challenge is that the lack of a full understanding of the function of integrins and the role of integrins in diseases, in particular their roles in signaling.

5. Conclusions and future perspectives

In conclusion, ECM–integrin–CSK interactions are involved in rearrangements of cell body, axonal, dendritic, and astrocytic processes. However, the extent of integrin regulation of the synaptic events during neuronal diseases remains unclear. ECM–integrin–CSK-intracellular signaling interactions in the nervous system could play a major role in the responses of neurons and blood vessels to injury and repair. Both neuronal activity and vascular reactivity from tissue injury rapidly modulate integrin signaling with consequent modulation of ion channels and activities, the generation or exposure of new integrin ligands from limited degradation of extracellular matrix through increased extracellular proteases, and/or turnover of new integrins (Banes et al., 1995; Fukai et al., 1995; Jones et al., 1995; Gualandris et al., 1996; Hoffman et al., 1998a, 1998b; Momota et al., 1998; Wu et al., 1998; Endo et al., 1999; Davis et al., 2000; Wu et al., 2000; Davis et al., 2002; Waitkus-Edwards et al., 2002; Kramar et al., 2003; Lin et al., 2003; Martinez-Lemus et al., 2003; Bernard-Triolo et al., 2005; Gui et al., 2006; Wu et al., 2008b; Yang et al., 2010). Further studies are warranted to study how integrins modulate fast dynamic changes of ion channels such as GABAergic inhibition.

Integrins as receptors are attractive targets for pharmacological manipulation of integrin-based signaling. Specificity or selectivity is a challenge to develop therapies against integrins. To avoid or lower risk of side effects, drugs need to distinguish between integrins expressed in healthy and injured tissues, target to activated integrin receptor not only resting integrins, and compete for single \( \alpha \)\( \beta \) combinations, not only inhibiting discrete \( \alpha \) or \( \beta \) subunits. For instance, \( \alpha \)3\( \beta \)-3 and \( \alpha \)IIb\( \beta \)-3 share \( \beta \)-3 subunit and both recognize the RGD motif; drugs that inhibit \( \beta \)-3 subunit as \( \alpha \)3\( \beta \)-3 antagonist to inhibit angiogenesis will lead to side effects of bleeding from inhibiting \( \alpha \)IIb\( \beta \)-3.
Advances in structural characterization of integrin–ligand interactions will prove and has proved beneficial in the design and development of potent and selective inhibitors for each single combination. The small molecule antagonists that are small enough to block ligands from binding to α-subunit which determines ligand specificity, without interacting with β-subunit which activates signaling pathway, is also a potential strategy to achieve.

In neurological diseases, ECM proteins often degrade or denature. Development of integrin ligands that recognize these degraded or newly expressed ECM components is critical. In addition, it is not clear whether degraded integrin ligands serve as early biomarkers for diagnostic purposes. Recent reports support the utility of integrin ligands as potential biomarkers for diagnosis and treatment of tumor and cardiovascular diseases

References

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Conflict of interest statement

The authors declare that there are no conflicts of interest.


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