Chapter 9

Neuroblastoma Integrins

Shanique A. Young, Ryon Graf and
Dwayne G. Stupack

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/55991

1. Introduction

In the body, cells are surrounded and supported by an intricate network of glycoproteins and proteoglycans that make up a complex extracellular matrix, or ECM. Many constituents, such as collagen, laminin, and fibronectin, are locally produced within the tissues, where they act as physical scaffolds, growth factor depots, and points of anchorage [1]. The local rigidity and composition of the matrix also provide environmental cues that govern cell behavior.

The ECM surrounding cells can be considered in two broad classes. On one hand, there exists a 'physiologic' ECM, present in all tissues, that aids in structuring and maintaining homeostasis. Typical ECM components include several collagens and laminins, as well as proteoglycans. On the other hand, there is a provisional ECM that is deposited during wounding, hemostasis and tissue remodeling. This ECM is typically deposited, digested and replaced in a very dynamic manner, and contains proteins such as fibronectin, fibrin, vitronectin and even residual fragments of collagen and laminin. This type of ECM promotes tissue remodeling as well as cellular survival, proliferation and invasion. In both types of ECM, however, the diversity in the type and quantity of each individual ECM component present determines the physical properties of these tissues. In so doing, this modulates the mechanical forces sensed by cells that bind to the ECM, and provides yet another layer of information relayed to cells. This 'mechanosensation' requires integrins, receptors that can transmit extracellular forces to the actin cytoskeleton.

Although many classes of receptors can interact with components of the ECM, the integrins are regarded as the principle receptors mediating anchorage and attachment to the ECM [2]. The name integrin was derived from initial observations that these receptors permitted a realignment of the actin cytoskeleton to match that of an underlying ECM. Integrins are transmembrane glycoprotein receptors that are composed of a heterodimer of α and β subunits

[3]. There are 18 different α subunits and 8 β subunits, but there are a limited number of possible combinations that can form from these subunits. To date, at least 24 unique integrin complexes have been identified, each with its own binding specificity for different subsets of ligands (Figure 1). Cells will generally express only a limited number of integrins, perhaps 10 of these combinations. The particular repertoire of integrins expressed by a given cell varies, but is typically closely tied to a cell's particular extracellular microenvironment. Differences in integrin binding to a ligand can be subtle. For example, approximately one third of human integrins bind to an arginine-glycine-aspartic acid (RGD) sequence of amino acid residues, but this can be profoundly conformation specific, and thus not all 'RGD-binding' integrins are capable of binding all RGD sequences with appreciable affinity.

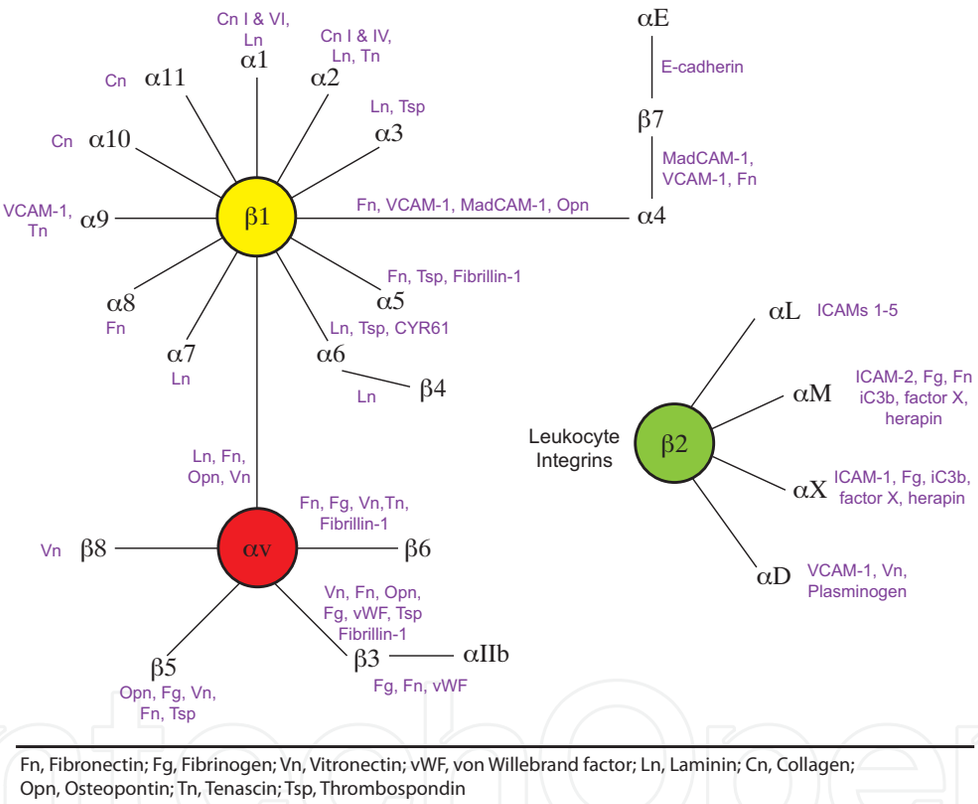

Figure 1. Integrin heterodimers and their ligands This diagram shows the 24 known heterodimers and their ligands. Integrin heterodimers are represented by an α and a β subunit connected by a black line. For example, the β1 subunit dimerizes with 12 different α subunits. The ligands for each heterodimer are written in purple.

1.1. Integrin structure

Each integrin is composed of a large extracellular region of 600-1000 amino acids, as well as a single transmembrane domain. The extracellular regions can be broadly thought of in terms of a head and stalk (leg/thigh) region; the head is the critical site for ligand binding and divalent cation binding, as well as heterodimerization between the α and β subunits. Most integrins

http://dx.doi.org/10.5772/55991

also have a small (~30-50 amino acid) cytosolic domain, with the singular exception being integrin β4, which has a large cytosolic domain that interacts with intermediate filaments [4]. Integrins are cysteine–rich proteins, and have extensive crosslinking within domains that stabilize domain structure. Thus, integrins appear at different sizes when analyzed on reducing and non-reducing gels, and detection of integrins by some antibodies may require either condition, depending upon the linearity or conformation dependence of an epitope.

The integrin extracellular domains are required for and sufficient to bind to ECM or to 'receptor-ligands' present on the surface of adjacent cells. However, the binding of integrins to their ligands is controlled by their conformation, which is influenced by the stalk and cytosolic regions of the molecule. Inactive integrins adapt a 'folded back' conformation at a region halfway up the stalk (at the 'genou,' or knee, between the thigh and leg). Active integrins are extended molecules with stalks separated, and intermediates between these states tend to have intermediate affinities for ligands. Integrin-ligand binding requires the presence of divalent cations, with a typical preference for manganese, magnesium and calcium, although the relative preference for optimal affinity varies among the different heterodimers. These divalent cations, and Mn^{+2} in particular, directly influence integrin conformation, stabilizing them in the extended and high affinity conformation (Figure 2).

With the exception of circulating hematopoietic cells, which tend to maintain their integrins in an inactive conformation, most cells that have been examined express both active and inactive integrins. Active integrins tend to form higher order clusters on the cell surface, which promotes their localization to sites of ligation. There, the integrins are further stabilized by interaction with ligand. The accumulation of integrins in these sites creates a 'Velcro-like' effect, with groups of integrins (rather than individual molecules) collaborating to strengthen anchorage and to induce downstream signaling points of extracellular matrix contact. This clustering effect is called integrin 'avidity' regulation, which is distinct from affinity. This permits the stable interaction with the ECM required for sustained cellular anchorage and signaling via the assembly of a 'focal adhesion complex' that accumulates proximal to the membrane.

The focal adhesion complex that forms is multifunctional, and is capable of signaling directly, scaffolding additional or alternative signals, and engaging the actin/myosin system. Thus, despite the absence of intrinsic kinase or proteolytic activity, integrins transform mechanical and chemical cues from the extracellular environment into intracellular signals that profoundly impact cell behavior and function.

The focal adhesion complex contains a complicated array of non-receptor kinases and adaptor proteins that mediate downstream signaling events. As will be discussed in more detail below, integrin effectors in the focal adhesion include diverse signaling elements such as: focal adhesion kinase (FAK), src kinase, cytoskeletal elements including talin, paxillin and vinculin, phosphoinositide 3 kinase, and small GTPases of the Ras and Rho families and their effectors [5, 6]. Importantly, as part of the clustering process, integrins tend to undergo lateral associations with other cell surface receptors such as the receptor tyrosine kinases, EGFR and VEGFR, which are important for other global cellular signaling events. This type of signaling, in which

the integrin ectodomain is ligated and transforms information from the extracellular environment into cues for cytosolic signaling events has been termed "outside-in signaling."

However, in some cases, signals from inside the cell result in changes in integrin conformation. These are typically associated with cytosolic proteins binding to the cytosolic domains of the integrins. This type of regulation of integrin conformation is called "inside-out signaling." Both types of signaling are important for understanding the role of integrins in normal tissues and in disease pathology.

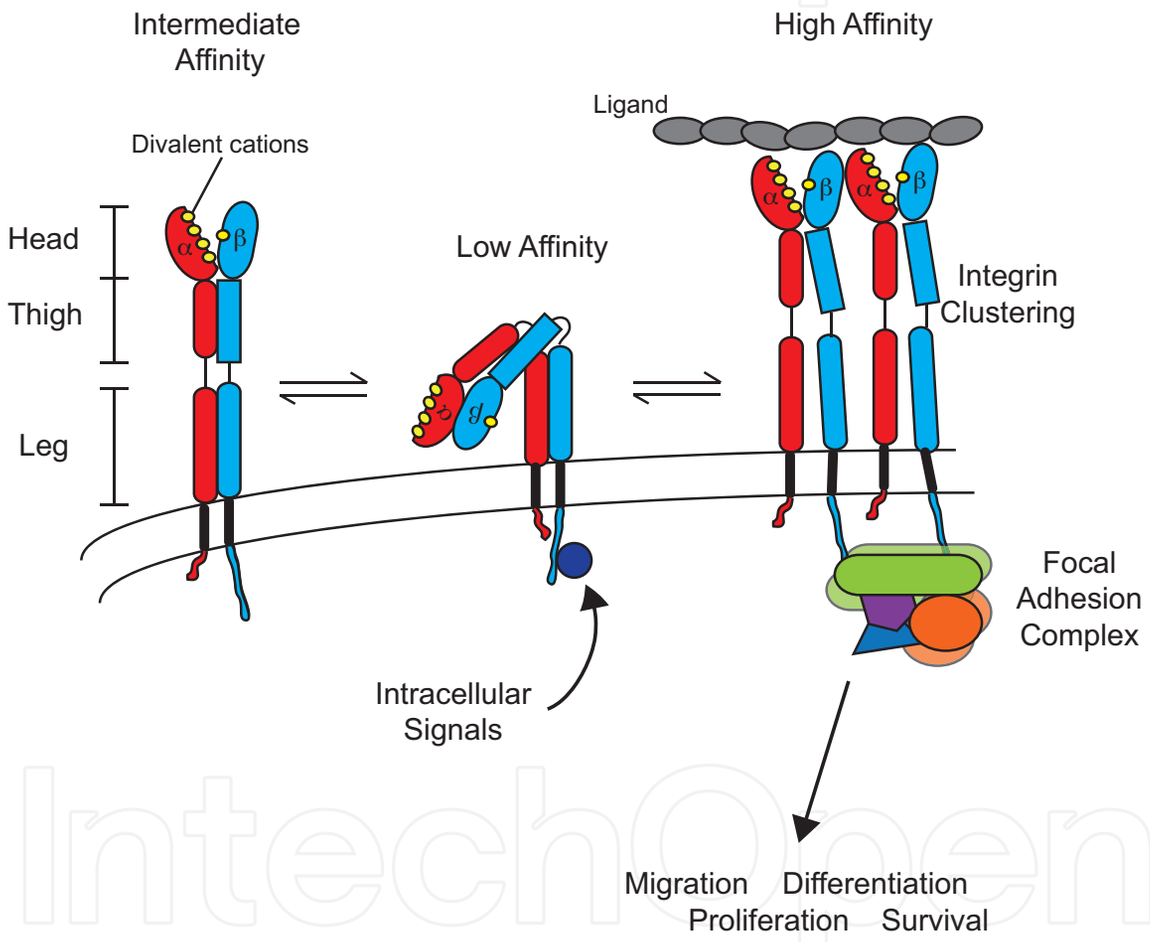

Figure 2. Integrin structure and activation Integrins are composed of a large extracellular domain and short intracellular tails (with the exception of the β4 tail). The extracellular domain comprises a head region and a stalk region, which includes the "thigh" and "leg" areas of the integrin. Ligand binding occurs at the head region and requires the presence of divalent cations such as manganese, magnesium, and calcium. Integrins on the cell surface can exist in a range of conformations that affect their affinity for ligand. In the low affinity conformation, the extracellular domain is folded back at the knee (between the thigh and leg areas) and the intracellular tails are clasped together. In the high affinity conformation the extracellular stalk is straight, the subunits are slightly separated and the tails shift apart as well. Conformations between these low and high affinity states confer intermediate affinity for ligand. Changes in conformation can be regulated by intracellular signaling events such as the binding of cytosolic proteins to integrin tails leading to integrin clustering, focal adhesion formation and further interaction of cytoskeletal proteins.

2. Integrins and development

2.1. Integrins in early development

The ability of cells to interact with their extracellular environment is crucial for most developmental processes. Consequently, it is perhaps not surprising that integrins, as mediators of the interplay between cells, the ECM and the microenvironment, have critical roles in early development. The early physiological relevance is evident in defects observed in murine genetic models lacking proper integrin function or expression. Overall, the loss of the β1, α5, and α4 subunits leads to an embryonic lethal phenotype. The loss of the αv or α3 subunits permits initial and subsequent development, but results in perinatal lethality. Other integrin subunits do not appear to be essential during development.

Nonetheless, loss, misregulation, or improper function of integrins can lead to other abnormalities [7]. (Table 1)

2.2. Integrins in nervous system development

The development of the nervous system is dependent on integrin function, in part, because it involves extensive migration of neuronal precursors which is mediated by integrins. During the process of neurulation, the neural crest forms in the region of the neural plate border. Upon formation of the neural tube, neural crest cells undergo an 'epithelial-to-mesenchymal-like transition' which permits them to move along migratory tracks. These tracks lead cells to a variety of destinations where they differentiate and help to form several different tissue types. During development, collagens, laminins, fibronectin and vitronectin are expressed along these migratory pathways [28]. Disruption of integrin-ligand binding inhibits neural crest cell migration and results in impaired function in the peripheral nervous system. Following the initial gross exodus of neurons from the neural crest, integrins also play other key roles in the development of the peripheral nervous system, including the establishment of Schwann cell polarity [29], neurite outgrowth [30, 31] and myelination [32].

In addition to the requirement for integrins to support migration, integrins are also important for arresting migration at the proper time and place. In the central nervous system, for example, the presence of the α6 and β1 subunits appears to serves as stop signals for neuronal cells when they reach a laminin rich region. This is critical for cortical plate formation. In the absence of these integrins, neuronal precursors migrating outward to the outermost layer of the cortical plate overshoot their destination and disrupt the cortical plate structure [33, 34].

2.3. Integrin expression in the dorsal root ganglion and in neuroblasts

Neuroblastoma is a tumor that is considered to arise from ganglion or pre-ganglion cells. To begin to understand the pathological roles of integrins in this disease, it is helpful to be familiar with the normal expression patterns of these receptors in neural crest cells and how that expression changes over time. Neural crest cells express subsets of integrins that allow them to adhere to the fibrillar proteins that line their migratory pathways. Truncal neural crest cells, which give rise to dorsal root ganglia, sympathetic ganglia, and the adrenal medulla express

Integrin subunit	Genetic Defect (KO)	Expressed on NB tumors	Notes
α1	Viable	Yes	Normal; [8]
α2	Viable	Yes	Abnormal mammary branching morphogenesis; [9]
α3	Perinatal lethality	Yes	Abnormal kidneys; [10]
α4	Lethal, by E14.5	Yes	Abnormal placenta and heart formation; [11]
α5	Lethal, E11	Yes	Abnormal mesoderm morphogenesis; [12]
α6	Perinatal lethality	Yes	Skin blistering; [13]
α7	Viable	Yes	Muscular dystrophy; [14]
α8	Perinatal lethality	No*	Abnormal kidneys and lungs; [15, 16]
α9	Perinatal lethality	No	Bilateral chylothorax; [17]
α10	Viable	No	Improper function of growth plate chondrocytes; [18]
α11	Viable	No	Dwarfism; [18]
αv	Perinatal lethality	Yes	Brain and bladder, hemorrhages; [19]
αL	Viable	No	Impaired leukocyte recruitment; [18]
αM	Viable	No	Impaired phagocytosis; obesity; [18]
αE	Viable	No	Inflammatory skin lesions; [18]
αIIb	Viable	No	Impaired platelet aggregation; [18]
β1	Lethal, E5.5	Yes	Abnormal mesoderm morphogenesis; [20]
β2	Viable	No	Impaired leukocyte recruitment; [21]
β3	Viable	Yes	Glanzmann's thrombasthenia; osteosclerotic; [22]
β4	Perinatal lethality	No	Skin blistering; [23]
β5	Viable	Yes	No apparent phenotype; [24]
β6	Viable	No	Macrophage infiltration in skin and lungs; [25]
β7	Viable	No	No gut-associated lymphoid tissue; [26]
β8	Lethal, E12 - birth	No*	Abnormal placenta; defects in neurovascular homeostasis; [27]

*Subunit found on neural crest cells but not yet reported on NB tumor cells

Table 1. Effects of Integrin Deletion in Murine Models

receptors for vitronectin (αvβ1, αvβ3, and αvβ5: [35]), laminin (α1β1, α3β1,: [36] [37]), and fibronectin and associated molecules (α4β1, α5β1, α8β1, αvβ1 and β8 integrin: [37], [38]. Antibody blockade of any one type of these integrins is unable to completely abolish cell migration, consistent with a multi-receptor and complex ligand system. However, in studies on avian truncal neural crest cells, the α3β1, α4β1, and αv integrins appear to be the most crucial to maintain migration [38]. In particular, inhibition of the interaction between α4β1 and

its ligands via blocking antibodies or ligand-mimicking peptides, leads to a marked reduction in neural crest cell migration [37].

As neural crest cells reach their target tissues and differentiate, their integrin expression changes. For example, neural crest cells do not express detectable levels of α6β1 until they differentiate into a peripheral nervous system cell type such as a Schwann cell precursor [39]. Conversely, neural crest cells express α1β1 but Schwann cell precursors do not [40, 41]. This induction of expression of one class of integrins while another is eliminated is not well understood, however, and further study will be required to elucidate additional neuroblast-specific integrin expression and function.

2.4. Integrins in vascular system development

Similarly, the formation of the vascular system relies heavily on integrin function. During vasculogenesis, or *de novo* formation of blood vessels, and angiogenesis, the growth of new vessels from pre-existing vasculature, integrins play essential roles in endothelial cell migration, adhesion to basement membranes and cell survival. Endothelial cells are known to express a large number of β1 integrin heterodimers including the α1 through α6 subunits as well as integrins α6β4, αvβ5, and αvβ3. The expression of different subsets of these integrins is dependent on the activation state of the endothelial cells. For example, integrins αvβ3 and α4β1 are primarily expressed on activated or angiogenic endothelial cells [42]. Knockout of integrin αv leads to perinatal lethality due to vessel malformation [15] and studies on the αvβ3 heterodimer show that it is essential for the survival of angiogenic endothelial cells [43]. In addition, knock-out of integrin α4 in mice is embryonic lethal by day 14.5 due to placental and cardiac defects [11], likely due to a lack of binding to the α4 ligand, vascular cell adhesion molecule-1 (VCAM-1) which is present on endothelial and smooth muscle cells.

The formation of the vasculature, and angiogenesis in particular, is of interest to scientists who study neuroblastoma, which is typically a highly angiogenic disease. Although a focus has been placed on the roles of integrins in development of the neuronal and vascular systems, the ability of integrins to regulate such a large array of cellular functions renders them essential for most, if not all, developmental processes. Their roles may be directly associated with their adhesion and motility-related functions, or with the ability of integrins to indirectly enhance the efficiency of other signaling pathways [44].

3. Integrin expression during tumorigenesis and tumor progression

3.1. Tumors exploit integrins for local invasion

As cells are transformed from a normal to malignant state, their integrin expression is modulated to support pathologic behaviors. In primary tumors, integrin signaling can impact cell growth, differentiation, and vascular infiltration and continues to be important as the cancer progresses through the stages of metastasis (Figure 3). The initial steps of the metastatic process involve the degradation and remodeling of extracellular matrix adjacent to primary

tumor cells, facilitating cancer cell migration into recruited blood vessels. This process is termed local invasion. Usually, for local invasion to begin, cells from the primary tumor shift from an epithelial or non-motile to a more mesenchymal phenotype. In addition, cells frequently create a pathway for themselves by inducing degradation of the matrix via enzymes such as matrix metalloproteases [45]. Integrins can regulate MMP expression and/or activity. For example, integrin $\alpha 2\beta 1$ is a positive regulator of MMP-1 expression [46, 47].

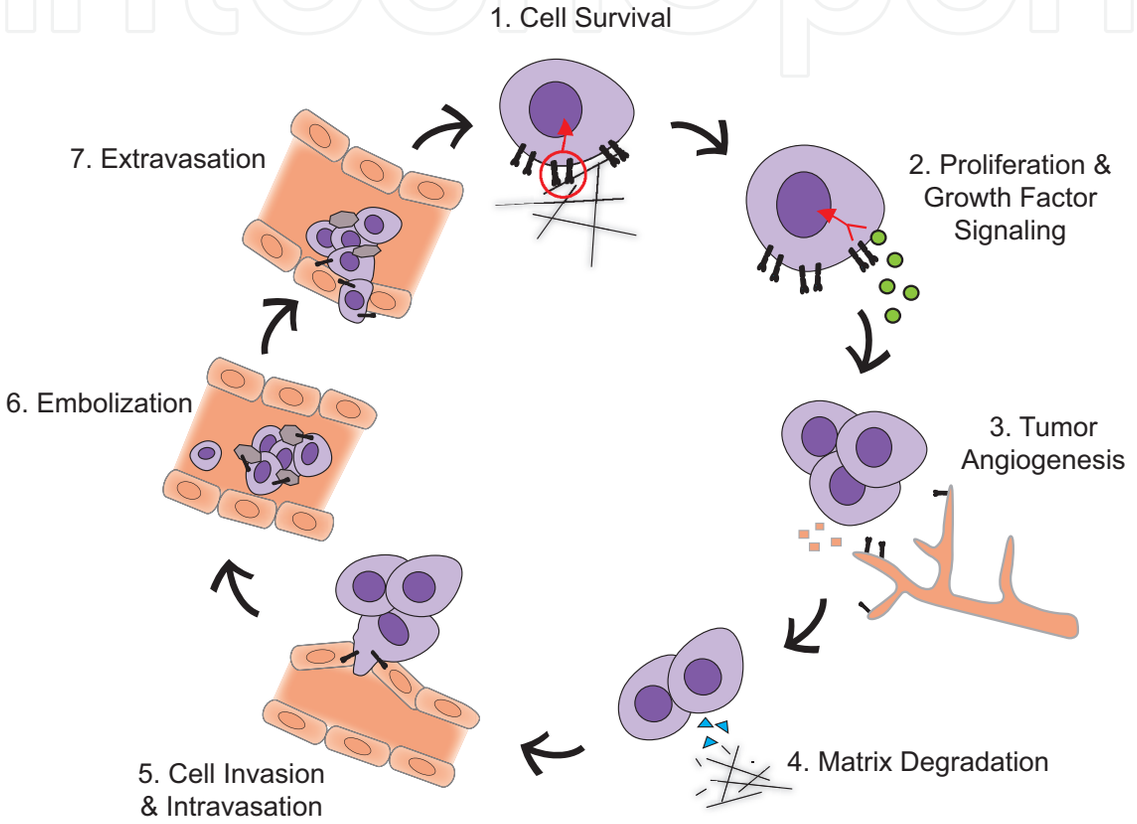

Figure 3. Roles Played by integrins in cancer progression Integrins play key roles in each phase of cancer progression. 1. Ligation of integrins promotes cell survival 2. Co-signaling with growth factor receptors impacts cell proliferation 3. Endothelial cell integrins are important for tumor angiogenesis 4. Integrins modulate the expression of proteolytic enzymes such as matrix metalloproteinases, which play a role in matrix degradation during tumor cell invasion 5. Integrins are required for migration during invasion and binding to endothelial cells during intravasation (entry into the vasculature) 6. In circulation, tumor cells interact with platelets and leukocytes via integrins and form cell emboli that can lodge in capillary beds of distant tissues 7. Binding of tumor cell integrins such as α4β1 to endothelial VCAM-1 can then promote extravasation of tumor cells into surrounding tissues.

3.2. Integrins, tumor metastasis, and tissue tropism

For many types of cancer, metastasizing cells spread to a specific subset of secondary locations for establishment of metastatic nodules. This phenomenon, termed tissue tropism, has historically been explained by two major theories. The "seed and soil" hypothesis proposed

by Stephen Paget in 1889 followed his observation of tissue-specific patterns of tumor metastasis in 735 breast cancer patients. Paget noted that the pattern of organs bearing metastases was not random, and suggested that certain tumor types preferentially metastasized to compatible environments [48]. He proposed that 'seeds' of tumors required compatible 'soil' to take root and grow. An alternative theory, by Ewing, suggests that tissue tropism is simply due to mechanical forces and circulatory patterns [49], and that tissue tropism results from this. These are not absolutely exclusive theories, and it is reasonable that blood flow patterns are important for the initial distribution of circulating tumor cells, while the propensity to invade, grow and survive may be dependent on the presence of the appropriate integrin ligands as well as other pro-survival factors.

Though there have been no studies specifically linking integrins to site-specific metastasis in neuroblastoma, integrins have been shown to play a role in tissue tropism. The primary sites of neuroblastoma metastasis are bone marrow, bone, lymph node and liver. In general, certain integrins have been linked to metastasis to these sites. For instance, integrin $\alpha 4\beta 1$ can promote homing to the bone [50] and has been shown to enhance bone metastasis in melanoma [51]. This effect may be due to expression of VCAM-1 on bone marrow stromal cells. Integrin $\alpha 4\beta 1$ may also promote lymphatic metastasis by enhancing binding to VCAM-1 present on lymphatic endothelial cells [52]. Integrin $\alpha 2\beta 1$ is associated with enhanced liver metastasis. This is potentially due to its binding to collagen type IV expressed in liver sinusoids [53].

3.3. Indirect roles for integrins in metastasis

Since the metastatic cascade involves several steps, including local tumor invasion, intravasation, survival in the lymphatics/blood stream, extravasation, invasion into the new tissue parenchyma and growth and establishment of metastatic nodules, there are many opportunities for integrins to facilitate this process. The role of integrins in local invasion is clear. Once cells gain entry into the vasculature, integrins are important for cell-cell and cell-platelet adhesion leading to increased formation of cell emboli [54] and subsequent lodging in capillary beds. Integrins are also important for the endothelial transmigration that follows. At the site of distant metastasis, the microenvironment and composition of the extracellular matrix may be different from that of the native tissue of the invading tumor cells. Here, the balance of ligated and unligated integrins impacts cell behavior and survival, as discussed in Section 4.

The shedding of gangliosides also impacts neuroblastoma metastasis. Gangliosides are glycosphingolipids with one or more sialic acids linked to them. In circulation, gangliosides are associated with lipoproteins. There are several different types of gangliosides that are classified based on the number of associated sialic acids. Some of these gangliosides, such as G_{M3}, are normally present in circulation. Conversely, elevated levels of circulating G_{D2}, a disialoganglioside, have been found in neuroblastoma patients and its concentration is inversely related to progression-free survival. Shedding of gangliosides enhances integrin $\alpha 2\beta 1$-dependent platelet activation, leading to platelet aggregation, and increased adhesion to vascular basement membranes [55]. These events can enhance tumor cell embolization impacting the occurrence of cells lodging in capillary beds and invading into surrounding tissue.

Finally, it is worth noting that at any phase of tumor progression, cancer cells must evade the immune system. Some T-cell lysis mechanisms are dependent on integrin expression. For instance, binding of T-cell integrin LFA-1 (αLβ2) to its ligands ICAM-1 on tumor cells is important in CD3-mediated T-cell lysis [56, 57]. Of note, ICAM expression on neuroblastoma cells is associated with increased susceptibility to lymphokine-activated killer (LAK) cell lysis following interferon gamma treatment [58].

3.4. Trends in integrin expression with neuroblastoma stage and grade

Since integrins impact cell differentiation and invasion, there has been an interest in linking the expression of subsets of integrins with a particular tumor stage, or more appropriately, with tumor 'risk.' Key risk predictors to date have been established by the Children's Oncology Group, and include status of the MYCN gene, the pathology of the tumor according to guidelines established by Shimada [59], and in some cases the relative ploidy of the tumor. Since integrins are associated with neuronal cell developmental stages and activities, it is reasonable that integrin expression could offer insights into tumor activities.

In pioneering studies, using 45 clinical samples, Favrot et al. showed that the α2 and α6 subunits were associated with low grade, well-differentiated neuroblastoma samples. The finding is consistent with observations of normal 'neural crest cell to neuronal' differentiation. The β1 subunit was expressed on all samples while the α5 subunit was not expressed on any samples examined. Samples expressing the α4, αv, β3, and β4 subunits revealed no N-Myc amplification, and were associated with a good prognosis. In addition, expression of α4 and β4 subunits was found selectively on Schwannian stromal cells [60].

Conversely, more recent studies have found that many neuroblastoma cell lines express integrin α4 and that α4 expression is associated with increased tumor stage (stages 3 and 4) in clinical samples [61]. At least on cell lines, integrin α5β1 also appears to be expressed [45] and integrin αvβ3 has been described to be present on some malignant neuroblastomas [62]. In addition, by flow cytometry, our lab consistently observes low levels of integrin αvβ5 on established neuroblastoma cell lines, although whether this is a tissue culture adaptation or reflects actual expression *in situ* remains unclear. Indeed, neuroblasts exhibit significant plasticity, and although integrins may be associated with specific stages of neuroblastoma, or specific developmental states where transformation of the neuroblast initially occurred, an alternative hypothesis is that neuroblastoma may retain the capacity to alter their relative integrin expression, and that this type of plasticity may itself be a malignancy factor.

Neuroblastomas fall into three common morphological/adhesive categories when grown *in vitro*: S (Substrate adherent), N (Neuroblastic), and I (Intermediate) types [63, 64]. These different types are sometimes ascribed to a particular cell line, though in many cases a cell line may contain cells of all three types. Studies using tissue culture cell lines have shown that, relative to S-type, N-type neuroblastomas exhibit decreased expression of β1 integrin and greater expression of αvβ3, and are more migratory *in vitro*. However, the expression of αvβ3 on these cells is still relatively low, at least when one compares with tissues well known to express αvβ3, such as angiogenic endothelium or melanoma. N-type cells also form more colonies in soft agar and are more tumorigenic when implanted in mice than S-type, which are

rarely able to form xenograft tumors [65]. S-type cells express fibronectin; it is therefore not surprising that they represent the group of neuroblastoma that express α5β1integrin, the fibronectin receptor [66].

The third type of cells is the 'intermediate cells.' Noted as potential 'cancer stem cells' as early as 1989 by Ross and colleagues, these cells look like an intermediate between the N and S types via diverse measures including phase contrast microscopy, intermediate filament expression, tyrosine hydroxylase activity, and norepinephrine uptake [64]. Consistent with being a tumor stem-like cell (or tumor initiating cell), I-type cells are by far the most tumorigenic in mice and in *in vitro* surrogate assays of tumor formation. Treatment with 13-cis retinoic acid or 5-bromo-2'-deoxyuridine can differentiate I-type cells into N-type or S-type, respectively. Retinoic acid has significant effects on integrins, consistent with changes seen during neuronal differentiation, and can differentiate some neuroblastomas into a benign growth-arrested state [67]. Clinically, retinoic acid has also been demonstrated to improve event free and overall survival in a long-term follow-up on a large cohort of neuroblastoma patients [68].

4. Signaling by integrins

In addition to key roles in cell anchorage and migration, integrin-mediated ligation of the extracellular matrix results in the initiation of signaling events exerting both local and cellular effects. Thus, the extracellular matrix encodes information via the local milieu of cell surface or diffusible factors presented to the cell (Figure 4). Most of these signals have been studied in rigorously defined systems *in vitro* with cell lines, rather than primary *in vivo* investigation.

4.1. Integrin ligation promotes the activation of the nonreceptor tyrosine kinases FAK and Src

Signaling that follows the ligation of integrins by extracellular matrix components can be studied by introducing suspended neuroblastoma cells to a surface coated with an extracellular matrix component, such as fibronectin. This results in cell attachment and spreading. Concurrent with these events, phosphorylation is observed on cytosolic nonreceptor tyrosine kinases like FAK (tyrosine residue 397) and Src (tyrosine residue 418), which indicate activation of the tyrosine kinases. At least some of this activity is physically present in the integrin associated focal adhesion complex, and these kinases can be co-purified with integrins from this complex.

FAK and Src can associate with each other and with an array of cytosolic adaptor proteins and other effectors. For example, FAK can associate with the cytoskeletal adaptor protein talin, which also binds to integrins. The adhesion of NB7 neuroblastoma cells to fibronectin or collagen has been shown to promote co-association of these molecules together in a complex with the protease calpain. Calpain in turn cleaves talin in a cell-adhesion dependent manner, which faciliates more rapid turn over of the focal adhesion, and promotes neuroblastoma cell migration. The same cleavage is observed in other neuroblastoma cells, including NB5 and NB16, suggesting it may be a conserved pathway [69].

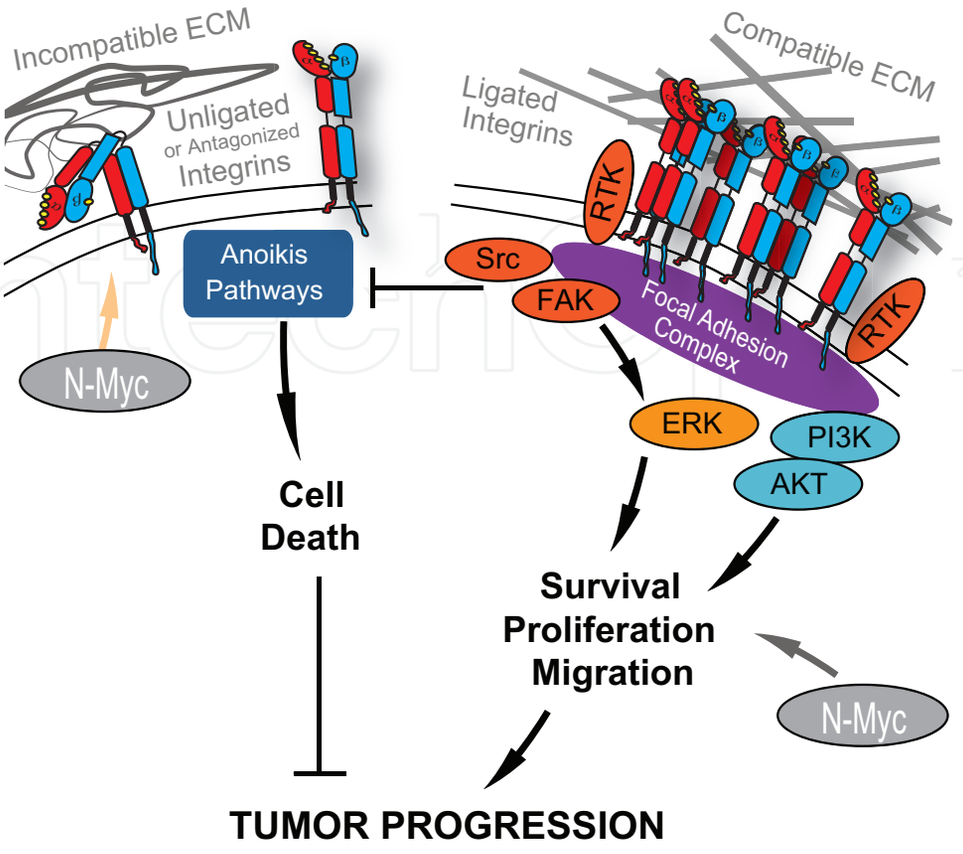

Figure 4. Signaling Pathways Downstream of Integrins The continued survival of a single cell and its progeny in the wrong environment can disrupt the homeostasis of the tissue that contains them. Thus, the impetus of individual cells to live or die is critical for the continued homeostasis of an organism. Recognition of compatible ECM promotes stable ligation and clustering of integrins, as well as assembly of the heterogeneous and dynamic focal adhesion complex. Signaling from integrins and focal adhesion-associated receptor tyrosine kinases (RTK) leads to downstream pro-survival signaling pathways such as the PI3K /AKT and Erk axes. By contrast, the presence of an incompatible ECM or of unligated or antagonized integrins promotes cell death via anoikis pathways, including integrin-mediated death. N-Myc exerts pleiotropic effects via transcription (or inhibition thereof) of many downstream genes, enhancing proliferation and survival, and attenuating the expression of integrins, and therefore decreasing anoikis signaling.

FAK also associates with Grb2 and SoS [70], key regulators of Ras-GTP mediated activation of the Raf/MEK/ERK pathway of MAP kinase signaling. This pathway helps to drive proliferation of the tumor cells, and may account for adhesion-based induction of cyclin E in neuroblastoma (and other) cells [71]. FAK is perhaps best known for its capacity to support and promote integrin-mediated cell migration on an ECM, and performs this function in neuroblastoma cells as well, although this appears to be integrin specific [61]. For example, integrin $\alpha 5\beta 1$ activates FAK and uses this kinase for migration, while integrin $\alpha 4\beta 1$ migration is dependent upon the non-receptor kinase Src. Both integrins can bind to a fibronectin substrate, thus the particular integrin ligated can have an impact on the cells' response. Other effects of specific integrin ligation have been reported in non-neuroblastoma cell lines, such as the FAK and $\alpha 5\beta 1$-induced expression of the pro-survival gene Bcl-2 [72]. Thus, signals from FAK can play a role in regulating cell survival in an ECM and integrin-dependent manner.

http://dx.doi.org/10.5772/55991

4.2. Integrin activation of the phosphoinositide 3′ kinase signaling axis

Integrins stabilized and ligated to correct ECM promote signaling via class I phosphoinisotol-3 kinases (PI3K). PI3K's are a family of lipid bound kinases found at the cell membrane or intracellular endosomes, and can promote cell motility, intracellular trafficking and survival. Among the four class I PI3K's, neuroblastoma tend to express P110α and p110β, with the latter more likely to be associated with N-Myc expressing tumors. Nonetheless, P110γ and p110δ are also sometimes detected [73]. Activation of the PI3K signaling axis promotes malignancy in numerous cancer cell lines and models of human cancer [74]. PI3K signaling also enhances turnover of pro-mitochondrial apoptotic proteins like Bad and promotes downstream pro-survival pathways, such as AKT and mTOR [75]. PTEN, a suppressor of PI3K, is frequently lost in cancer, although studies in neuroblastoma have shown a lesser degrees of loss, in the range of ~5% for homozygous deletion [76]. Mutations of PI3K that enhance kinase activity have been reported in other cancers [77], yet they have been proven to be infrequent in neuroblastoma [78]. Thus, the activity of PI3K appears to frequently depend upon extrinsic regulatory factors, mediated by receptor tyrosine kinases (eg., IGFR-1, ALK) and integrins.

Given the lack of effective therapies for malignant neuroblastoma, it is perhaps not surprising that the PI3K pathway is being pursued for pharmacological intervention [79]. In neuroblastoma, inhibition of PI3K has been demonstrated to decrease migration and survival of tumor cells *in vitro*, and inhibit tumor growth *in vivo* [80, 81]. The efficacy of pharmacological PI3K inhibition may be enhanced by combining a pro-drug with an RGDS peptide to target the agent to tumor sites [81]. The relative affinity for this linear peptide for integrin, however, is quite low, and it is improbable that enhanced efficacy is due to direct action on integrins, rather, it is likely due to improved pharmacokinetics associated with the targeting peptide.

4.3. Interplay between integrins and signature neuroblastoma signaling pathways

N-Myc is a transcription factor normally expressed during early lymphocyte development and in embryonic brain and kidney tissues [82], and is critical for survival of neural crest-derived neurons [83]. Amplification of greater than ten copies of the *MYCN* gene has long been recognized as a strong negative prognostic indicator of outcome in neuroblastoma [84]. N-Myc interacts with integrins in an antagonistic manner; while N-Myc seems to increase expression of FAK, it has also been shown to down-regulate the expression of integrins such as α3β1 and α1β1 [85-88]. Transcriptional analysis of the β3 and αv promoters have revealed negative transcriptional regulatory elements in their promoters by the closely related c-Myc [76], suggesting why αvβ3 is not highly expressed in neuroblastoma relative to other tumors. In fact, the loss of integrin expression may be important for survival in specific circumstances, particularly among tumors that retain intrinsic apoptotic capacity, as discussed below.

ALK is a tyrosine kinase that is expressed largely during development within the nervous system. ALK belongs to the 'insulin-like tyrosine kinase' family of receptors that is frequently upregulated or subject to oncogenic mutation in neuroblastoma [89]. Signaling by tyrosine kinases generally requires integrin ligation [5], activating downstream targets (such as FAK, Src, PI3K etc.). This suggests that there is an intrinsic requirement for ECM adhesion to permit a tumor to 'leverage' amplified ALK. However, mutant forms of ALK also exist, particularly

a F1174 mutation that drives neuroblastoma malignancy cooperatively with MYCN. In this case, it is unclear whether integrin-mediated adhesion is actually required for cell proliferation, although it is likely to enhance signaling in keeping with the rationale described above. MYCN also leads to increased expression of a close ALK relative, insulin-like growth factor I receptor (IGF-IR). In this case, crosstalk between IGF-IR and integrins is also observed [90].

4.4. Integrins and cell survival signaling

Cells that lose anchorage for extended periods of time will typically undergo apoptosis. This phenomenon encompasses one aspect of anoikis (gr., homelessness), a phenomenon wherein a cell that finds itself in an inappropriate environment is signaled to undergo apoptosis. However, there is no 'central cell death pathway' associated with anoikis, and in fact many different pathways have been validated in the literature. This underscores the critical need for cell adhesion. One anoikis pathway is focused on the activation of caspase-9. Although many neuroblastomas lose expression of one copy of caspase-9 (as many are LOH1p21), this does not appear to impact the capacity of caspase-9 to activate [91]. Antagonism of b1 integrins on differentiated neuroblastoma, but not undifferentiated, promotes this apoptotic pathway [92].

Integrin-mediated death is an anoikis pathway in which the presence of unligated, or antagonized, integrins on the cell surface promote cell death via the activation of caspase-8. Neuroblastoma avoid this death pathway via several mechanisms. First, the amplification of MYCN can lead to an overall decrease in integrin expression, which lowers the capacity of the pathway to trigger. Secondly, stage III and IV neuroblastoma tend to methylate, delete, or disrupt the caspase-8 gene [93, 94], preventing the triggering of the apoptotic pathway, and this results in a survival advantage *in vitro* and a metastasis advantage *in vivo*. Finally, neuroblastoma that are seeded as individual cells in an 'inappropriate' three dimensional matrix will tend to either die or, within only a couple days, find each other and form small cell clusters. These islands of cells promote their own survival and can persist, although they are sometimes surrounded by apoptotic bodies as errant progeny try to migrate away from the original cell mass.

Opposing the induction of death by unligated or antagonized integrins, it is worth noting that a cell that has a robust interaction with the ECM is more resistant to certain insults than others, and integrin ligation has been linked to chemo and radiation resistance. Mechanistically, this is likely to result from remodeling of the ECM, combined with transcriptional alterations of survival promoting genes such as Bcl-2 family members, IAPs and others. However, direct effects, such as maturation-inhibiting phosphorylation of procaspase-8, cannot be excluded from contributing to this effect [95, 96].

5. Specific integrins in neuroblastoma progression

5.1. Integrin $\alpha v\beta 3$

Integrin $\alpha v\beta 3$ is the most 'promiscuous' member of the integrin family, in that it binds a variety of different RGD conformations, and thus binds to ligands that include vitronectin, fibronectin,

fibrinogen, von Willebrand factor and others. Gladson et al. found that αv was present in all tumors they examined regardless of stage. While αvβ1 and αvβ5 heterodimers were found in normal adrenal tissues and ganglioneuroblastomas which exhibit lower levels of dissemination, the αvβ3 integrin was found to be expressed in highly metastatic, undifferentiated neuroblastomas [62]. By contrast, we observe only very low levels of integrin αvβ3 on our neuroblastoma specimens relative to melanoma or cultured endothelial cells, which express robust levels of αvβ3. However, it remains possible that the techniques originally used by Gladson were simply very sensitive and detected this modest but important level of integrin expression. Indeed, αvβ3 is, in some systems, a stem cell marker, and this may reflect the advanced stage and poor prognosis of her positive cohort.

In addition, on a variety of tumor cells, αvβ3 expression has been demonstrated to promote tumor progression by its ability to bind to a wide array of different ligands, facilitating anchorage and invasion. Integrin αvβ3 also stimulates MMP activity, promotes the activation of receptor and non-receptor tyrosine kinases including src, and the release of growth factors such as TGF that promote tumor response. This vascularization provides the growing tumor with the nutrients it needs and brings tumor cells proximal to vessels, which may facilitate invasion and metastasis. As previously mentioned, αvβ3 is also expressed on angiogenic endothelial cells where it promotes cell survival and migration. One study showed that there is higher β3 expression on invasive and metastatic melanomas than on noninvasive melanomas [97], although the levels demonstrated in these cases appear to be logarithmically higher than those seen on neuroblastoma cell lines [98].

5.2. Integrin α4β1 and tumor spread

Integrin α4β1 is primarily known as a trafficking integrin, as it is present on most leukocytes. Binding to its ligand VCAM-1, present on activated endothelial cells, enhances the transendothelial migration of white blood cells into surrounding tissues. Cancer cells that express α4β1 acquire this same enhanced trafficking potential and show increased tumor cell arrest in circulation and increased extravasation and colony formation. α4β1 may also enhance invasion and metastasis through promotion of angiogenesis and lymphangiogenesis [99, 100]. In [97], α4β1 expression was found on 40% of invasive and metastatic melanomas, although not on non-malignant melanocytes.

It is important to note that, though the expression of α4β1 can indeed promote extravasation, the overall role of integrin α4β1 in tumor progression and metastasis is highly controversial and is dependent on the level of expression and the phase of tumor progression. For example, high α4β1 expression in some primary tumors can enhance homotypic cell-cell adhesion [101], preventing cells from breaking away from the tumor and invading into surrounding tissues [102]. In addition, α4β1 expression can lead to a reduction in MMPs and impair the ability of the cells to degrade the matrix and create a pathway for invasion [103]. If cells do successfully metastasize to distant sites, α4β1 expression may promote or inhibit metastatic growth depending on the microenvironment.

6. Drugs that target integrins

The involvement of integrins in multiple stages of tumor progression makes them attractive therapeutic targets. Inhibition of integrin signaling can be achieved using several approaches including blocking ligand binding, preventing the formation of functional focal adhesion complexes and disrupting integrin association with the cytoskeleton. Because the structure of integrins has been extensively studied and because having an extracellular target eliminates the challenges of intracellular delivery, the most common approach has been to target the integrin ligand-binding site. This has been accomplished using blocking antibodies, cyclic and ligand-mimicking peptides, small molecule antagonists and disintegrins [104] (Table 2).

Target	Antagonist	Type	Clinical Development
αvβ3	Vitaxin	humanized antibody	Phase II trials
	CNTO 95	humanized antibody	Phase II trials
	c7E3 (Abciximab)	Chimeric mouse- human antibody	FDA approved (1994) for use in percutaneous coronary intervention (PCI)
	Cilengitide	cyclic peptide	Phase III trials for glioblastoma multiforme; Phase II trials for melanoma, glioma, and SCCHN; Phase I trials for NSCLC
	L000845704	Small molecule	Phase I trials
	SB273005	Small molecule	Pre-clinical animal studies
α4β1	Natalizumab	humanized antibody	FDA approved (1994) for treatment of multiple sclerosis and Crohn's disease
	MLN-00002	human antibody	Phase II trials
	Firategrast	small molecule	Phase II trials
αIIbβ3	c7E3 (Abciximab)	Chimeric mouse- human antibody	FDA approved (1994) for use in percutaneous coronary intervention (PCI)
	Eptifibatide	cyclic peptide	FDA approved (1998) for use in patients with acute coronary syndrome or undergoing PCI
	Tirofiban	small molecule	FDA approved in 1999
α5β1	Volociximab	chimeric human-mouse antibody	Phase II trials in melanoma, pancreatic cancer, and NSCLC
	JSM6427	small molecule	Phase I trials
α2β1	Rhodocetin	disintegrin	Pre-clinical

Table 2. Drugs that Target Integrins

6.1. Integrin αv

The primary rationale for targeting integrin αvβ3 in cancer is to reduce primary tumor growth and metastasis via nutrient deprivation due to inhibition of tumor angiogenesis. Several αvβ3 antagonists have gone to clinical trials with the most notable being cilengitide. Cilengitide is a cyclic peptide containing the RGD integrin-binding motif. It inhibits both αvβ3 and αvβ5. Cilengitide produces both anti-angiogenic and anti-tumor effects through inhibition of VEGF stimulation and FAK-Src and Erk signaling, respectively [105]. *In vitro*, cilengitide reduces cell growth and survival and inhibits endothelial and tumor cell migration. In clinical trials, cilengitide has been evaluated as a single agent and in combination with radiation, DNA-alkylating agents and gemcitibine. Importantly, cilengitide in combination with radiotherapy and temozolomide (a DNA-alkylating agent) has reached phase III trials in glioblastoma multiforme patients. Other small molecule antagonists are in development for noncancer indications.

6.2. Integrin α4

The integrin α4 subunit is predominantly expressed in lymphocytes and leukocytes and supports endothelial transmigration of these cells via binding to VCAM-1. Consequently, α4 is important for immune function and has been targeted in diseases such as multiple sclerosis (MS), Crohn's disease and asthma that are characterized by excessive inflammation or an improper immune response. Natalizumab, the only FDA approved α4 antagonist, is a humanized mouse monoclonal antibody that binds both α4 heterodimers. The use of natalizumab was successful in clinical trials in MS [106, 107] and Crohn's disease [108] with the exception of rare cases of progressive multi-focal leukoencephalopathy (PML) caused by reactivation of latent JC virus associated with immunosuppression [109]. Unfortunately, this side effect was detrimental enough to lead to limitation of the use of natalizumab to patients who are unresponsive to other treatments. Other α4 antagonists under clinical evaluation include MLN-00002 (human α4β7 antibody), firategrast and IVL745 (small molecules: [104]). Though the rationale for the use of most α4 antagonists is to reduce excessive infiltration of immune cells, these therapies have the potential for use against cancer cells that exploit α4 for tumor cell extravasation. The success of targeting α4 in cancer will depend on the ability to minimize immunosuppression or to indirectly impair α4 function via downstream targets.

6.3. Integrin αIIbβ3

Integrin αIIbβ3 is also a frequently targeted integrin. This heterodimer is expressed selectively on platelets and megakaryocytes and is mostly known for its role in blood coagulation. Antagonists of this receptor are primarily employed in diseases such as stroke, sickle cell anemia and acute coronary syndromes [104].

7. Summary and considerations

Integrins are a unique group of receptors that provide anchorage, mediate cell migration and invasion, and signal via cell survival and proliferation pathways. Aptly named,

integrins integrate extracellular cues with intracellular signaling and serve to regulate many cellular processes that are mediated by other receptors, such as receptor tyrosine kinases. The importance of integrins in cancer development of the nervous system is well established; it seems inevitable therefore that they play a major role in neuroblastoma progression. In fact, integrin expression has been linked to malignancy in neuroblastoma, possibly due to alterations in invasiveness and the ability to evade cell death in foreign tissue environments. Aggressive disease may modulate integrin expression (i.e. N-Myc).

Targeting integrins has shown great clinical promise. By inhibiting ligand binding, many antagonists successfully disrupt cellular connections to the extracellular environment and pro-survival pathways that are necessary for tumor progression. As we continue to learn more about the downstream signaling activity of integrin receptors, we can also explore more therapeutic avenues against these targets, attacking the problem from both sides. However, the logical use of integrin antagonists in complex, multi-agent regimens is lacking. Given the synergy of integrins with signaling through receptor tyrosine kinases and in the induction of susceptibility to apoptosis, this is where one would suspect that these relatively non-toxic agents would have their greatest impact.

Though clinical studies of integrin-targeted drugs in neuroblastoma have not been performed, *in vitro* antagonism has been shown to decrease cell survival, migration and invasion. Despite these characteristics, integrin-targeted drugs are well tolerated. Given that current treatment for neuroblastoma still has a significant failure rate, the addition of new, low toxicity adjuncts to current treatment regimens seems a logical step forward. In the future, an increased understanding of the roles of specific integrins in neuroblastoma has the potential to provide better prognostic information regarding disease course, while targeting integrins, perhaps in combination with other targeted therapies as a cocktail addition to standard chemotherapy approaches, may lead to increased effectiveness in managing this disease.

Author details

Shanique A. Young, Ryon Graf and Dwayne G. Stupack*

*Address all correspondence to: dstupack@ucsd.edu

Reproductive Medicine Department, UCSD Moores Cancer Center, La Jolla, California, USA

References

[1] Hynes RO. The extracellular matrix: not just pretty fibrils. Science. 2009;326(5957): 1216-9. PubMed PMID: 19965464. eng.

[2] Ingber D. Integrins as mechanochemical transducers. Curr Opin Cell Biol. 1991 Oct; 3(5):841-8. PubMed PMID: 1931084. eng.

[3] Hynes RO. Integrins: a family of cell surface receptors. Cell. 1987 Feb;48(4):549-54. PubMed PMID: 3028640. eng.

[4] Stepp MA, Spurr-Michaud S, Tisdale A, Elwell J, Gipson IK. Alpha 6 beta 4 integrin heterodimer is a component of hemidesmosomes. Proc Natl Acad Sci U S A. 1990 Nov;87(22):8970-4. PubMed PMID: 2247472. Pubmed Central PMCID: PMC55082. Epub 1990/11/01. eng.

[5] Schlaepfer DD, Hunter T. Integrin signalling and tyrosine phosphorylation: just the FAKs? Trends Cell Biol. 1998 Apr;8(4):151-7. PubMed PMID: 9695829. eng.

[6] Juliano RL, Reddig P, Alahari S, Edin M, Howe A, Aplin A. Integrin regulation of cell signalling and motility. Biochem Soc Trans. 2004 Jun;32(Pt3):443-6. PubMed PMID: 15157156. eng.

[7] Beauvais-Jouneau A, Thiery JP. Multiple roles for integrins during development. Biol Cell. 1997 Mar;89(1):5-11. PubMed PMID: 9297778. eng.

[8] Gardner H, Kreidberg J, Koteliansky V, Jaenisch R. Deletion of integrin alpha 1 by homologous recombination permits normal murine development but gives rise to a specific deficit in cell adhesion. Dev Biol. 1996 May;175(2):301-13. PubMed PMID: 8626034. eng.

[9] Chen J, Diacovo TG, Grenache DG, Santoro SA, Zutter MM. The alpha(2) integrin subunit-deficient mouse: a multifaceted phenotype including defects of branching morphogenesis and hemostasis. Am J Pathol. 2002;161(1):337-44. PubMed PMID: 12107118. eng.

[10] Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, et al. Alpha 3 beta 1 integrin has a crucial role in kidney and lung organogenesis. Development. 1996 Nov;122(11):3537-47. PubMed PMID: 8951069. eng.

[11] Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. Development. 1995 Feb;121(2): 549-60. PubMed PMID: 7539359. eng.

[12] Yang JT, Rayburn H, Hynes RO. Embryonic mesodermal defects in alpha 5 integrin-deficient mice. Development. 1993 Dec;119(4):1093-105. PubMed PMID: 7508365. eng.

[13] Georges-Labouesse E, Messaddeq N, Yehia G, Cadalbert L, Dierich A, Le Meur M. Absence of integrin alpha 6 leads to epidermolysis bullosa and neonatal death in mice. Nat Genet. 1996 Jul;13(3):370-3. PubMed PMID: 8673141. eng.

[14] Mayer U, Saher G, Fässler R, Bornemann A, Echtermeyer F, von der Mark H, et al. Absence of integrin alpha 7 causes a novel form of muscular dystrophy. Nat Genet. 1997 Nov;17(3):318-23. PubMed PMID: 9354797. eng.

[15] Fässler R, Georges-Labouesse E, Hirsch E. Genetic analyses of integrin function in mice. Curr Opin Cell Biol. 1996 Oct;8(5):641-6. PubMed PMID: 8939651. eng.

[16] Hartner A, Haas C, Amann K, Sterzel RB. Aspects of the renal phenotype of adult alpha8 integrin-deficient mice. Nephrol Dial Transplant. 2002;17 Suppl 9:71-2. PubMed PMID: 12386295. Epub 2002/10/19. eng.

[17] Huang XZ, Wu JF, Ferrando R, Lee JH, Wang YL, Farese RV, Jr., et al. Fatal bilateral chylothorax in mice lacking the integrin alpha9beta1. Mol Cell Biol. 2000 Jul;20(14): 5208-15. PubMed PMID: 10866676. Pubmed Central PMCID: PMC85969. Epub 2000/06/24. eng.

[18] Srichai MB, Zent R. Integrin Structure and Function. In: Zent R, Pozzi A, editors. Cell-Extracellular Matrix Interactions in Cancer. 1st Edition ed. New York: Springer; 2009.

[19] McCarty JH, Monahan-Earley RA, Brown LF, Keller M, Gerhardt H, Rubin K, et al. Defective associations between blood vessels and brain parenchyma lead to cerebral hemorrhage in mice lacking alphav integrins. Mol Cell Biol. 2002 Nov;22(21):7667-77. PubMed PMID: 12370313. Pubmed Central PMCID: PMC135679. Epub 2002/10/09. eng.

[20] Fässler R, Meyer M. Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. 1995 Aug;9(15):1896-908. PubMed PMID: 7544313. eng.

[21] Wilson RW, Ballantyne CM, Smith CW, Montgomery C, Bradley A, O'Brien WE, et al. Gene targeting yields a CD18-mutant mouse for study of inflammation. J Immunol. 1993 Aug;151(3):1571-8. PubMed PMID: 8101543. eng.

[22] McHugh KP, Hodivala-Dilke K, Zheng MH, Namba N, Lam J, Novack D, et al. Mice lacking beta3 integrins are osteosclerotic because of dysfunctional osteoclasts. J Clin Invest. 2000 Feb;105(4):433-40. PubMed PMID: 10683372. Pubmed Central PMCID: PMC289172. eng.

[23] Dowling J, Yu QC, Fuchs E. Beta4 integrin is required for hemidesmosome formation, cell adhesion and cell survival. J Cell Biol. 1996 Jul;134(2):559-72. PubMed PMID: 8707838. Pubmed Central PMCID: PMC2120864. eng.

[24] Huang X, Griffiths M, Wu J, Farese Jr. RV, Sheppard D. Normal Development, Wound Healing, and Adenovirus Susceptibility in β5-Deficient Mice. 2000 2000-02-01. en.

[25] Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, et al. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. J Cell Biol. 1996 May;133(4):921-8. PubMed PMID: 8666675. Pubmed Central PMCID: PMC2120829. eng.

[26] Wagner N, Löhler J, Kunkel EJ, Ley K, Leung E, Krissansen G, et al. Critical role for beta7 integrins in formation of the gut-associated lymphoid tissue. Nature. 1996 Jul; 382(6589):366-70. PubMed PMID: 8684468. eng.

[27] Mobley AK, Tchaicha JH, Shin J, Hossain MG, McCarty JH. Beta8 integrin regulates neurogenesis and neurovascular homeostasis in the adult brain. J Cell Sci. 2009;122(Pt 11):1842-51. PubMed PMID: 19461074. eng.

[28] Erickson CA, Perris R. The role of cell-cell and cell-matrix interactions in the morphogenesis of the neural crest. Dev Biol. 1993 Sep;159(1):60-74. PubMed PMID: 8365575. eng.

[29] Bartlett Bungee M. Schwann cell regulation of extracellular matrix biosynthesis and assembly. In: Dyck PJ, Thomas PK, Griffin J, Low P, Poduslo J, editors. Peripheral Neuropathy. Philadelphia, PA: Saunders; 1993.

[30] Kuhn TB, Schmidt MF, Kater SB. Laminin and fibronectin guideposts signal sustained but opposite effects to passing growth cones. Neuron. 1995 Feb;14(2):275-85. PubMed PMID: 7531986. eng.

[31] Luckenbill-Edds L, Kaiser CA, Rodgers TR, Powell DD. Localization of the 110 kDa receptor for laminin in brains of embryonic and postnatal mice. Cell Tissue Res. 1995 Feb;279(2):371-7. PubMed PMID: 7895274. eng.

[32] Fernandez-Valle C, Gwynn L, Wood PM, Carbonetto S, Bunge MB. Anti-beta 1 integrin antibody inhibits Schwann cell myelination. J Neurobiol. 1994 Oct;25(10):1207-26. PubMed PMID: 7529296. eng.

[33] Georges-Labouesse E, Mark M, Messaddeq N, Gansmüller A. Essential role of alpha 6 integrins in cortical and retinal lamination. Curr Biol. 1998 Aug;8(17):983-6. PubMed PMID: 9742403. eng.

[34] Graus-Porta D, Blaess S, Senften M, Littlewood-Evans A, Damsky C, Huang Z, et al. Beta1-class integrins regulate the development of laminae and folia in the cerebral and cerebellar cortex. Neuron. 2001 Aug;31(3):367-79. PubMed PMID: 11516395. eng.

[35] Delannet M, Martin F, Bossy B, Cheresh DA, Reichardt LF, Duband JL. Specific roles of the alpha V beta 1, alpha V beta 3 and alpha V beta 5 integrins in avian neural crest cell adhesion and migration on vitronectin. Development. 1994 Sep;120(9): 2687-702. PubMed PMID: 7525179. Pubmed Central PMCID: PMC2710119. eng.

[36] Duband JL, Belkin AM, Syfrig J, Thiery JP, Koteliansky VE. Expression of alpha 1 integrin, a laminin-collagen receptor, during myogenesis and neurogenesis in the avian embryo. Development. 1992 Nov;116(3):585-600. PubMed PMID: 1337741. eng.

[37] Kil SH, Krull CE, Cann G, Clegg D, Bronner-Fraser M. The alpha4 subunit of integrin is important for neural crest cell migration. Dev Biol. 1998 Oct;202(1):29-42. PubMed PMID: 9758701. eng.

[38] Testaz S, Delannet M, Duband J. Adhesion and migration of avian neural crest cells on fibronectin require the cooperating activities of multiple integrins of the (beta)1 and (beta)3 families. J Cell Sci. 1999 Dec;112 (Pt 24):4715-28. PubMed PMID: 10574719. eng.

[39] Bronner-Fraser M, Artinger M, Muschler J, Horwitz AF. Developmentally regulated expression of alpha 6 integrin in avian embryos. Development. 1992 May;115(1): 197-211. PubMed PMID: 1638980. eng.

[40] Perris R. The extracellular matrix in neural crest-cell migration. Trends Neurosci. 1997 Jan;20(1):23-31. PubMed PMID: 9004416. eng.

[41] Stewart HJ, Turner D, Jessen KR, Mirsky R. Expression and regulation of alpha1beta1 integrin in Schwann cells. J Neurobiol. 1997 Dec;33(7):914-28. PubMed PMID: 9407013. eng.

[42] Stupack DG, Cheresh DA. Integrins and angiogenesis. Current topics in developmental biology. 2004;64:207-38. PubMed PMID: 15563949. Epub 2004/11/27. eng.

[43] Brooks PC, Montgomery AM, Rosenfeld M, Reisfeld RA, Hu T, Klier G, et al. Integrin alpha v beta 3 antagonists promote tumor regression by inducing apoptosis of angiogenic blood vessels. Cell. 1994 Dec;79(7):1157-64. PubMed PMID: 7528107. eng.

[44] Bökel C, Brown NH. Integrins in development: moving on, responding to, and sticking to the extracellular matrix. Dev Cell. 2002 Sep;3(3):311-21. PubMed PMID: 12361595. eng.

[45] Kähäri NR. Matrix Metalloproteinases in Cancer Cell Invasion. 2000 2000. en.

[46] Znoyko I, Trojanowska M, Reuben A. Collagen binding alpha2beta1 and alpha1beta1 integrins play contrasting roles in regulation of Ets-1 expression in human liver myofibroblasts. Mol Cell Biochem. 2006 Jan;282(1-2):89-99. PubMed PMID: 16317516. Epub 2005/12/01. eng.

[47] Riikonen T, Westermarck J, Koivisto L, Broberg A, Kahari VM, Heino J. Integrin alpha 2 beta 1 is a positive regulator of collagenase (MMP-1) and collagen alpha 1(I) gene expression. J Biol Chem. 1995 Jun 2;270(22):13548-52. PubMed PMID: 7768957. Epub 1995/06/02. eng.

[48] Paget S. The distribution of secondary growths in cancer of the breast. 1889. Cancer Metastasis Rev. 1989 Aug;8(2):98-101. PubMed PMID: 2673568. eng.

[49] Ewing J. A treatise on tumors. Philadelphia, PA: W.B. Saunders Company; 1928.

[50] Kumar S, Ponnazhagan S. Bone homing of mesenchymal stem cells by ectopic alpha 4 integrin expression. FASEB J. 2007 Dec;21(14):3917-27. PubMed PMID: 17622670. eng.

[51] Matsuura N, Puzon-McLaughlin W, Irie A, Morikawa Y, Kakudo K, Takada Y. Induction of experimental bone metastasis in mice by transfection of integrin alpha 4

beta 1 into tumor cells. Am J Pathol. 1996 Jan;148(1):55-61. PubMed PMID: 8546226. Pubmed Central PMCID: PMC1861618. eng.

[52] Rebhun RB, Cheng H, Gershenwald JE, Fan D, Fidler IJ, Langley RR. Constitutive expression of the alpha4 integrin correlates with tumorigenicity and lymph node metastasis of the B16 murine melanoma. Neoplasia. 2010 Feb;12(2):173-82. PubMed PMID: 20126475. Pubmed Central PMCID: PMC2814355. eng.

[53] Yoshimura K, Meckel KF, Laird LS, Chia CY, Park JJ, Olino KL, et al. Integrin alpha2 mediates selective metastasis to the liver. Cancer Res. 2009 Sep;69(18):7320-8. PubMed PMID: 19738067. eng.

[54] Ruoslahti E, Giancotti FG. Integrins and tumor cell dissemination. Cancer Cells. 1989 Dec;1(4):119-26. PubMed PMID: 2701367. eng.

[55] Jabbar AA, Kazarian T, Hakobyan N, Valentino LA. Gangliosides promote platelet adhesion and facilitate neuroblastoma cell adhesion under dynamic conditions simulating blood flow. Pediatr Blood Cancer. 2006 Mar;46(3):292-9. PubMed PMID: 16317740. Epub 2005/12/01. eng.

[56] Anichini A, Mortarini R, Supino R, Parmiani G. Human melanoma cells with high susceptibility to cell-mediated lysis can be identified on the basis of ICAM-1 phenotype, VLA profile and invasive ability. Int J Cancer. 1990 Sep;46(3):508-15. PubMed PMID: 1975567. eng.

[57] Braakman E, Goedegebuure PS, Vreugdenhil RJ, Segal DM, Shaw S, Bolhuis RL. ICAM- melanoma cells are relatively resistant to CD3-mediated T-cell lysis. Int J Cancer. 1990 Sep;46(3):475-80. PubMed PMID: 1975566. eng.

[58] Naganuma H, Kiessling R, Patarroyo M, Hansson M, Handgretinger R, Gronberg A. Increased susceptibility of IFN-gamma-treated neuroblastoma cells to lysis by lymphokine-activated killer cells: participation of ICAM-1 induction on target cells. Int J Cancer. 1991 Feb 20;47(4):527-32. PubMed PMID: 1671670. Epub 1991/02/20. eng.

[59] Shimada H. The International Neuroblastoma Pathology Classification. Pathologica. 2003 Oct;95(5):240-1. PubMed PMID: 14988991. eng.

[60] Favrot MC, Combaret V, Goillot E, Lutz P, Frappaz D, Thiesse P, et al. Expression of integrin receptors on 45 clinical neuroblastoma specimens. Int J Cancer. 1991 Sep; 49(3):347-55. PubMed PMID: 1917132. eng.

[61] Wu L, Bernard-Trifilo JA, Lim Y, Lim ST, Mitra SK, Uryu S, et al. Distinct FAK-Src activation events promote alpha5beta1 and alpha4beta1 integrin-stimulated neuroblastoma cell motility. Oncogene. 2008 Feb 28;27(10):1439-48. PubMed PMID: 17828307. Pubmed Central PMCID: 2593630. Epub 2007/09/11. eng.

[62] Gladson CL, Hancock S, Arnold MM, Faye-Petersen OM, Castleberry RP, Kelly DR. Stage-specific expression of integrin alphaVbeta3 in neuroblastic tumors. Am J Path-

ol. 1996 May;148(5):1423-34. PubMed PMID: 8623914. Pubmed Central PMCID: PMC1861568. eng.

[63] Ross RA, Spengler BA. Human neuroblastoma stem cells. Semin Cancer Biol. 2007 Jun;17(3):241-7. PubMed PMID: 16839774. eng.

[64] Ciccarone V, Spengler BA, Meyers MB, Biedler JL, Ross RA. Phenotypic diversification in human neuroblastoma cells: expression of distinct neural crest lineages. Cancer Res. 1989 Jan;49(1):219-25. PubMed PMID: 2535691. eng.

[65] Spengler BA, Lazarova DL, Ross RA, Biedler JL. Cell lineage and differentiation state are primary determinants of MYCN gene expression and malignant potential in human neuroblastoma cells. Oncol Res. 1997;9(9):467-76. PubMed PMID: 9495452. eng.

[66] Meyer A, van Golen CM, Kim B, van Golen KL, Feldman EL. Integrin expression regulates neuroblastoma attachment and migration. Neoplasia. 2004 2004 Jul-Aug;6(4): 332-42. PubMed PMID: 15256055. Pubmed Central PMCID: PMC1502107. eng.

[67] Hadjidaniel MD, Reynolds CP. Antagonism of cytotoxic chemotherapy in neuroblastoma cell lines by 13-cis-retinoic acid is mediated by the antiapoptotic Bcl-2 family proteins. Mol Cancer Ther. 2010 Dec;9(12):3164-74. PubMed PMID: 21159604. Pubmed Central PMCID: PMC3182269. eng.

[68] Matthay KK, Reynolds CP, Seeger RC, Shimada H, Adkins ES, Haas-Kogan D, et al. Long-term results for children with high-risk neuroblastoma treated on a randomized trial of myeloablative therapy followed by 13-cis-retinoic acid: a children's oncology group study. J Clin Oncol. 2009 Mar;27(7):1007-13. PubMed PMID: 19171716. Pubmed Central PMCID: PMC2738615. eng.

[69] Barbero S, Mielgo A, Torres V, Teitz T, Shields DJ, Mikolon D, et al. Caspase-8 association with the focal adhesion complex promotes tumor cell migration and metastasis. Cancer research. 2009 May 1;69(9):3755-63. PubMed PMID: 19383910. Pubmed Central PMCID: 2684981. Epub 2009/04/23. eng.

[70] Schlaepfer DD, Hanks SK, Hunter T, van der Geer P. Integrin-mediated signal transduction linked to Ras pathway by GRB2 binding to focal adhesion kinase. Nature. 1994 1994 Dec 22-29;372(6508):786-91. PubMed PMID: 7997267. eng.

[71] Hulleman E, Bijvelt JJ, Verkleij AJ, Verrips CT, Boonstra J. Integrin signaling at the M/G1 transition induces expression of cyclin E. Exp Cell Res. 1999 Dec;253(2):422-31. PubMed PMID: 10585265. eng.

[72] Matter ML, Ruoslahti E. A signaling pathway from the alpha5beta1 and alpha(v)beta3 integrins that elevates bcl-2 transcription. J Biol Chem. 2001 Jul;276(30):27757-63. PubMed PMID: 11333270. eng.

[73] Spitzenberg V, König C, Ulm S, Marone R, Röpke L, Müller JP, et al. Targeting PI3K in neuroblastoma. J Cancer Res Clin Oncol. 2010 Dec;136(12):1881-90. PubMed PMID: 20224967. eng.

[74] Osaki M, Oshimura M, Ito H. PI3K-Akt pathway: its functions and alterations in human cancer. Apoptosis. 2004 Nov;9(6):667-76. PubMed PMID: 15505410. eng.

[75] Scott PH, Brunn GJ, Kohn AD, Roth RA, Lawrence JC. Evidence of insulin-stimulated phosphorylation and activation of the mammalian target of rapamycin mediated by a protein kinase B signaling pathway. Proc Natl Acad Sci U S A. 1998 Jun;95(13): 7772-7. PubMed PMID: 9636226. Pubmed Central PMCID: PMC22753. eng.

[76] Muñoz J, Lázcoz P, Inda MM, Nistal M, Pestaña A, Encío IJ, et al. Homozygous deletion and expression of PTEN and DMBT1 in human primary neuroblastoma and cell lines. Int J Cancer. 2004 May;109(5):673-9. PubMed PMID: 14999773. eng.

[77] Hafsi S, Pezzino FM, Candido S, Ligresti G, Spandidos DA, Soua Z, et al. Gene alterations in the PI3K/PTEN/AKT pathway as a mechanism of drug-resistance (review). Int J Oncol. 2012 Mar;40(3):639-44. PubMed PMID: 22200790. eng.

[78] Dam V, Morgan BT, Mazanek P, Hogarty MD. Mutations in PIK3CA are infrequent in neuroblastoma. BMC Cancer. 2006;6:177. PubMed PMID: 16822308. Pubmed Central PMCID: PMC1533846. eng.

[79] Fulda S. The PI3K/Akt/mTOR pathway as therapeutic target in neuroblastoma. Curr Cancer Drug Targets. 2009 Sep;9(6):729-37. PubMed PMID: 19754357. eng.

[80] Opel D, Naumann I, Schneider M, Bertele D, Debatin KM, Fulda S. Targeting aberrant PI3K/Akt activation by PI103 restores sensitivity to TRAIL-induced apoptosis in neuroblastoma. Clin Cancer Res. 2011 May;17(10):3233-47. PubMed PMID: 21355080. eng.

[81] Peirce SK, Findley HW, Prince C, Dasgupta A, Cooper T, Durden DL. The PI-3 kinase-Akt-MDM2-survivin signaling axis in high-risk neuroblastoma: a target for PI-3 kinase inhibitor intervention. Cancer chemotherapy and pharmacology. 2011 Aug; 68(2):325-35. PubMed PMID: 20972874. Pubmed Central PMCID: 3143317. Epub 2010/10/26. eng.

[82] Hurlin PJ. N-Myc functions in transcription and development. Birth Defects Res C Embryo Today. 2005 Dec;75(4):340-52. PubMed PMID: 16425253. eng.

[83] Sawai S, Shimono A, Wakamatsu Y, Palmes C, Hanaoka K, Kondoh H. Defects of embryonic organogenesis resulting from targeted disruption of the N-myc gene in the mouse. Development. 1993 Apr;117(4):1445-55. PubMed PMID: 8404543. eng.

[84] Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science. 1984 Jun;224(4653):1121-4. PubMed PMID: 6719137. eng.

[85] Judware R, Lechner R, Culp LA. Inverse expressions of the N-myc oncogene and beta 1 integrin in human neuroblastoma: relationships to disease progression in a nude mouse model system. Clin Exp Metastasis. 1995 Mar;13(2):123-33. PubMed PMID: 7533687. eng.

[86] Judware R, Culp LA. Over-expression of transfected N-myc oncogene in human SKNSH neuroblastoma cells down-regulates expression of beta 1 integrin subunit. Oncogene. 1995 Dec;11(12):2599-607. PubMed PMID: 8545117. eng.

[87] Judware R, Culp LA. Concomitant down-regulation of expression of integrin subunits by N-myc in human neuroblastoma cells: differential regulation of alpha2, alpha3 and beta1. Oncogene. 1997 Mar;14(11):1341-50. PubMed PMID: 9178894. eng.

[88] Judware R, Culp LA. N-myc over-expression downregulates alpha3beta1 integrin expression in human Saos-2 osteosarcoma cells. Clin Exp Metastasis. 1997 May;15(3): 228-38. PubMed PMID: 9174124. eng.

[89] Azarova AM, Gautam G, George RE. Emerging importance of ALK in neuroblastoma. Semin Cancer Biol. 2011 Oct;21(4):267-75. PubMed PMID: 21945349. Pubmed Central PMCID: PMC3242371. eng.

[90] Zheng B, Clemmons DR. Blocking ligand occupancy of the alphaVbeta3 integrin inhibits insulin-like growth factor I signaling in vascular smooth muscle cells. Proc Natl Acad Sci U S A. 1998 Sep;95(19):11217-22. PubMed PMID: 9736716. Pubmed Central PMCID: PMC21622. eng.

[91] Teitz T, Lahti JM, Kidd VJ. Aggressive childhood neuroblastomas do not express caspase-8: an important component of programmed cell death. J Mol Med (Berl). 2001 Aug;79(8):428-36. PubMed PMID: 11511973. eng.

[92] Bonfoco E, Chen W, Paul R, Cheresh DA, Cooper NR. beta1 integrin antagonism on adherent, differentiated human neuroblastoma cells triggers an apoptotic signaling pathway. Neuroscience. 2000;101(4):1145-52. PubMed PMID: 11113363. eng.

[93] Teitz T, Wei T, Valentine MB, Vanin EF, Grenet J, Valentine VA, et al. Caspase 8 is deleted or silenced preferentially in childhood neuroblastomas with amplification of MYCN. Nat Med. 2000 May;6(5):529-35. PubMed PMID: 10802708. eng.

[94] Fulda S, Poremba C, Berwanger B, Häcker S, Eilers M, Christiansen H, et al. Loss of caspase-8 expression does not correlate with MYCN amplification, aggressive disease, or prognosis in neuroblastoma. Cancer Res. 2006 Oct;66(20):10016-23. PubMed PMID: 17047064. eng.

[95] Cursi S, Rufini A, Stagni V, Condo I, Matafora V, Bachi A, et al. Src kinase phosphorylates Caspase-8 on Tyr380: a novel mechanism of apoptosis suppression. The EMBO journal. 2006 May 3;25(9):1895-905. PubMed PMID: 16619028. Pubmed Central PMCID: 1456929. Epub 2006/04/19. eng.

[96] Keller N, Grütter MG, Zerbe O. Studies of the molecular mechanism of caspase-8 activation by solution NMR. Cell Death Differ. 2010 Apr;17(4):710-8. PubMed PMID: 19851329. eng.

[97] Albelda SM, Mette SA, Elder DE, Stewart R, Damjanovich L, Herlyn M, et al. Integrin distribution in malignant melanoma: association of the beta 3 subunit with tumor progression. Cancer Res. 1990 Oct;50(20):6757-64. PubMed PMID: 2208139. eng.

[98] Stupack DG, Teitz T, Potter MD, Mikolon D, Houghton PJ, Kidd VJ, et al. Potentiation of neuroblastoma metastasis by loss of caspase-8. Nature. 2006 Jan 5;439(7072): 95-9. PubMed PMID: 16397500. Epub 2006/01/07. eng.

[99] Garmy-Susini B, Jin H, Zhu Y, Sung RJ, Hwang R, Varner J. Integrin alpha4beta1-VCAM-1-mediated adhesion between endothelial and mural cells is required for blood vessel maturation. J Clin Invest. 2005 Jun;115(6):1542-51. PubMed PMID: 15902308. Pubmed Central PMCID: PMC1088016. eng.

[100] Garmy-Susini B, Varner JA. Roles of integrins in tumor angiogenesis and lymphangiogenesis. Lymphat Res Biol. 2008;6(3-4):155-63. PubMed PMID: 19093788. Pubmed Central PMCID: PMC2837754. eng.

[101] Qian F, Vaux DL, Weissman IL. Expression of the integrin alpha 4 beta 1 on melanoma cells can inhibit the invasive stage of metastasis formation. Cell. 1994 May;77(3): 335-47. PubMed PMID: 8181055. eng.

[102] Beauvais A, Erickson CA, Goins T, Craig SE, Humphries MJ, Thiery JP, et al. Changes in the fibronectin-specific integrin expression pattern modify the migratory behavior of sarcoma S180 cells in vitro and in the embryonic environment. J Cell Biol. 1995 Feb;128(4):699-713. PubMed PMID: 7532177. Pubmed Central PMCID: PMC2199886. eng.

[103] Huhtala P, Humphries MJ, McCarthy JB, Tremble PM, Werb Z, Damsky CH. Cooperative signaling by alpha 5 beta 1 and alpha 4 beta 1 integrins regulates metalloproteinase gene expression in fibroblasts adhering to fibronectin. J Cell Biol. 1995 May; 129(3):867-79. PubMed PMID: 7537277. Pubmed Central PMCID: PMC2120442. eng.

[104] Millard M, Odde S, Neamati N. Integrin targeted therapeutics. Theranostics. 2011;1:154-88. PubMed PMID: 21547158. Pubmed Central PMCID: PMC3086618. eng.

[105] Oliveira-Ferrer L, Hauschild J, Fiedler W, Bokemeyer C, Nippgen J, Celik I, et al. Cilengitide induces cellular detachment and apoptosis in endothelial and glioma cells mediated by inhibition of FAK/src/AKT pathway. J Exp Clin Cancer Res. 2008;27:86. PubMed PMID: 19114005. Pubmed Central PMCID: PMC2648308. eng.

[106] Dalton CM, Miszkiel KA, Barker GJ, MacManus DG, Pepple TI, Panzara M, et al. Effect of natalizumab on conversion of gadolinium enhancing lesions to T1 hypointense lesions in relapsing multiple sclerosis. J Neurol. 2004 Apr;251(4):407-13. PubMed PMID: 15083284. eng.

[107] Havrdova E, Galetta S, Hutchinson M, Stefoski D, Bates D, Polman CH, et al. Effect of natalizumab on clinical and radiological disease activity in multiple sclerosis: a retrospective analysis of the Natalizumab Safety and Efficacy in Relapsing-Remitting

Multiple Sclerosis (AFFIRM) study. Lancet Neurol. 2009 Mar;8(3):254-60. PubMed PMID: 19201654. eng.

[108] Targan SR, Feagan BG, Fedorak RN, Lashner BA, Panaccione R, Present DH, et al. Natalizumab for the treatment of active Crohn's disease: results of the ENCORE Trial. Gastroenterology. 2007 May;132(5):1672-83. PubMed PMID: 17484865. eng.

[109] Lindå H, von Heijne A, Major EO, Ryschkewitsch C, Berg J, Olsson T, et al. Progressive multifocal leukoencephalopathy after natalizumab monotherapy. N Engl J Med. 2009 Sep;361(11):1081-7. PubMed PMID: 19741229. eng.