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REVIEW

The Roles of Integrin α5β1 in Human Cancer

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Abstract: Cell adhesion to the extracellular matrix has important roles in tissue integrity and human health. Integrins are heterodimeric cell surface receptors that are composed by two non-covalently linked alpha and beta subunits that mainly participate in the interaction of cell-cell adhesion and cell-extracellular matrix and regulate cell motility, adhesion, differentiation, migration, proliferation, etc. In mammals, there have been eighteen α subunits and 8 β subunits and so far 24 distinct types of αβ integrin heterodimers have been identified in humans. Integrin α5β1, also known as the fibronectin receptor, is a heterodimer with α5 and β1 subunits and has emerged as an essential mediator in many human carcinomas. Integrin α5β1 alteration is closely linked to the progression of several types of human cancers, including cell proliferation, angiogenesis, tumor metastasis, and cancerogenesis. In this review, we will introduce the functions of integrin α5β1 in cancer progression and also explore its regulatory mechanisms. Additionally, the potential clinical applications as a target for cancer imaging and therapy are discussed. Collectively, the information reviewed here may increase the understanding of integrin α5β1 as a potential therapeutic target for cancer.

Keywords: integrin α5β1, prognostic indicator, tumorigenesis, molecular target

Introduction

Integrins, the family of heterodimeric cell surface receptors that are expressed in most cells including pericytes, endothelial cells, fibroblasts, and tumor cells, which have emerged as important regulators for providing both mechanical engagement of cell to extracellular matrix, and generation of signals that are implicated in various diseases such as autoimmune diseases, deleterious embryonic development, cardiovascular diseases and cancer malignancies.[1,2] In mammals, eighteen α subunits and 8 β subunits form at least 24 distinct types of αβ integrin heterodimers, which play as true receptors of tissue and organ-specific ligands.[3] Both α and β subunits possess a large extracellular domain, a small cytoplasmic tail, and a transmembrane domain.[4] The extracellular domains act as the cells sense and respond to the microenvironment cues such as adhesion proteins and growth factors.[3] The cytoplasmic tail is linked to the actin cytoskeleton and intracellular signaling pathways such as Src family kinase, focal adhesion kinase (FAK), and mitogen-activated protein kinase (MAPK), as well as protein kinase B (AKT).[5] Notably, integrins have been received attention as important regulators in mediating the hallmarks that characterize human cancers, including cell proliferation, metastasis, immune evasion, tumor angiogenesis, and resistance to chemotherapy and radiotherapy.

Integrin α5β1was firstly reported in the 1992s and was the only known α5 integrin.[6] Upon binding to the ligand, the cytoplasmic tails of integrin α5β1 bind to cytoskeleton and then drive reorganization of the cytoskeleton through the

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intracellular signaling pathway, that is, the "outside-in" signaling pathway.[7,8] Integrin α5β1-mediated intracellular signals can in turn activate extracellular regions and assist ECM assembly, that is, the "inside-out" signaling pathway.[9,10] This two-way signaling pathway contributes to various biological activities, such as cell adhesion, migration, and survival.[11] And these behaviors can be mediated by arginine-glycine-aspartate (RGD) peptides, specific antibodies, as well as the surface glycosylation.[12] Integrin α5β1 has been defined as a proangiogenic factor involve in regulating tumor angiogenesis by interacting with the Vascular Endothelial Growth Factor Receptor (VEGFR) and angiopoietin-Tie systems.[13] Moreover, the important roles in tumorigenesis, tumor metastasis, and resistance to chemotherapy and radiotherapy have been highlighted for integrin α5β1.[14] In this review, we focus on the recent findings and important progress to summarize the roles and related mechanisms of integrin α5β1, and discuss the potential strategies targeting integrin α5β1 for improving cancer patient's outcomes.

Structure, Regulation, Ligands and Functions of Integrin α5β1

Structural Domains of Integrin α5β1

Integrin α5β1, as a member of the integrin family, is a heterodimer composed by two subunits, α5 and β1, and both are necessary for complete biological functions.[15] The human integrin alpha 5 gene (*ITGA5*) encodes the α5 subunit and is localized at 12q11. The extracellular domain of α5 subunit has a thigh domain and a β-propeller domain, which is responsible for the recognition of the RGD motifs on the fibronectin and fibrinogen.[16] The integrin beta 1 gene (*ITGB1*) has been proved to reside in chromosome 10p11.2, and the extracellular part of this subunit is made up of a plexin/semaphorin/integrin (PSI) domain, a hybrid domain, a βI domain (with a metal ion-dependent adhesion site [MIDAS] structure), and four EGF like domains.[17] The interactions of integrin α5β1 and its extracellular ligands are dependent on the MIDAS structure and divalent cations.[18] A recent crystal structure of α5β1 integrin has demonstrated that the specific residue (Asp154) could be used to distinguish α5 from other α subunits as its strong preference for fibronectin over other RGD ligands, and also indicated that Ca^{2+} is an important cation for ligand-binding of α5β1 integrin.[14,16]

Regulation of Integrin α5β1

miRNA Pathways Contribute to Post-Transcriptional Regulation of Integrin α5β1

miRNAs are a class of small endogenous noncoding RNAs and have emerged as important molecules that post-transcriptionally regulate gene expression.[19] The expression of α5β1integrin is determined by the transcriptional activity of *ITGA5* and *ITGB1* genes. The 3′-untranslated region of the *ITGA5* and *ITGB1*mRNA possess several miRNA target sequences. Recently, some miRNAs were reported to regulate the expression of integrin α5β1 under various pathological conditions. For example, both integrin α5 and β1 were directly targeted by miR-17 in ovarian cancer cell lines, and forced expression of miR-17 significantly blocked adhesion and invasion of ovarian cancer cells by inhibiting the expression of integrin α5 and β1.[20] In addition, miR-23a directly targeted the 3′ UTR of High Mobility Group Nucleosomal Binding Domain 2 (*HMGN2*) mRNA, which was involved in integrin α5β1 activation. miR-155 might also regulate integrin α5β1function by control of the expression and chromatin location of the integrin transcription suppressor-Nuclear Factor-I (*NFI*).[21] In breast cancer cells, miR-149 inhibited cancer cell metastasis by directly targeting GIT ArfGAP 1 (*GIT1*), which was responsible for the lysosome-mediated protein degradation of integrin α5β1.[22] Therefore, exploring the network of miRNAs and integrin α5β1 is essential to design strategies for better chemo-therapeutics.

Importance of Post-Translational Modifications (PTMs) in Regulation of Integrin α5β1

PTMs are made up of methylation, acetylation, phosphorylation, ubiquitination, neddylation, sulphation, sumoylation, prenylation, and glycosylation, which are the fundamental process for regulating the function of proteins, such as subcellular location, DNA-binding affinity, molecular half-life, and interactions with other proteins. N-Glycosylation of protein is considered to be as the most abundant PTM, and nearly 50% all known proteins are glycosylated in eukaryotes.[23] Integrins as the major glycan-carrying proteins, its complete biological functions rely on the N-Glycosylation modifications. Among the 24 human integrins, the functions of N-Glycosylation on integrin $\alpha_5\beta_1$ have been well characterized.[23,24] Gu and colleagues have identified several individual N-glycan sites in both α5 and β1subunits, which are critical for heterodimerization and biological functions of integrin α5β1.[25–28] For example, the N-glycan of β_1-N343 on the βI domain of β1 subunit is linked to integrin α5β1

activation. Loss of this glycan site led to the persistent activation of integrin α5β1;[29] The N-glycan sites on the I-like domain of the β1 subunit (β1S4-6) are important for integrin α5β1-mediated cell spreading and migration;[26] The N-glycosylation on the β-propeller domain of the α5 subunit (α5S3-5) are critical for the heterodimerization, and biological functions of integrin α5β1, as well as the formation of α5–syndecan-4 complex.[25,27] The site-11 N-glycosylation on calf domain of α5 subunit is also important for the α5-EGFR complex formation and the inhibitory effect on EGFR signaling.[28] Beyond glycosylation, ubiquitination of α5 subunit also plays an important role for integrin α5β1-mediated fibroblast migration.[30] Therefore, exploring the PTMs of integrin α5β1 is essential to understand the biological function and mechanism of integrin α5β1.

Potential Trafficking Machinery of Integrin α5β1

As transmembrane proteins, the transport of integrins to the cell surface is determined by the integrin trafficking machinery including exocytosis of integrins by vesicles and endocytosis of integrins at the plasma membrane. Integrin trafficking is considered to be an important regulator of cell adhesion and migration. The trafficking of α5β1 integrin is affected by several proteins such as CD151, Ras Homolog Family Member C (RhoC), Cytoskeleton-Associated Protein 4 (CKAP4), PTPRF Interacting Protein Alpha 1 (PPFIA1), Ankyrin-B, protein kinase B, syntaxins 3 and 4, Vesicle-Associated Membrane Protein 2 (VAMP2), Adaptor Protein, phosphotyrosine interacting with ph domain and leucine zipper 1 (APPL1), TGF-β type III receptor (TβRIII), and Neuropilin-2 (NRP-2).[31–41] For example, CD151 was functionally linked to integrin-mediated cell migration by control of the endocytosis and/or vesicular trafficking of α3β1, α5β1, and α6β1 integrins.[31] And mutation of the YXXφ endocytosis/sorting motif on the C-terminal cytoplasmic domain of CD151 significantly disrupted CD151-mediated cell migration.[31] In pancreatic carcinoma cells, RhoC over-expression enhanced integrin α5β1 internalization and trafficking, increasing the levels of α5β1 integrin at the cell surface and promoting cell metastasis.[32] TβRIII, a ubiquitous co-receptor for TGF-β, inhibited cell motility by control of β-arrestin2 dependent α5β1 internalization and recycling. In addition, TβRIII expression was significantly associated with α5 localization and overall survival in breast cancer patients.[40]

Extracellular Molecules Modulate Integrin α5β1

Extracellular molecules such as growth factor and receptors, cytokine and cytokine receptors, as well as extracellular matrix proteins are crucial regulatory factors affecting the biological activity and function of integrin α5β1. In cancer cells, epidermal growth factor (EGF) treatment could promote the p90RSK-dependent phosphorylation of filamin A (FLNa), which was responsible for the inactivation of integrin α5β1.[42] In addition, Dudvarski et al reported that epidermal growth factor-like protein 7 (EGFL7) elevated the levels of integrin α5β1 on the cellular surface and then promoted the fibronectin-induced angiogenesis in glioblastoma.[43] Interleukin 1β (IL-1β), an inflammatory cytokine, not only induced inflammatory but also increased integrin α5β1-dependent adhesion to fibronectin. Upon IL-1β treatment, the expression of α5 subunit increased and the active β1 subunit were relocated to focal contacts in the transformed human brain microvascular endothelial cells (THBMECs). And using α5-and β1-specific antibodies could remarkably inhibit the transmigration function under IL-1β-induced inflammatory conditions.[44] In basal-like breast cancer cells, CD44 elevated the expression and activity of β1 subunit, and also increased the expression of α5 subunit.[45] In addition, E-cadherin also associated with the expression and transcription activity of α5β1 in ovarian cancer cells. Sawada et al demonstrated that E-cadherin loss could increase α5β1 expression by regulating the FAK1/ERK1/MAPK signaling pathway.[46] Therefore, better understanding of the associations of extracellular molecules and α5β1 is essential for clinical therapy.

Ligands of α5β1

Integrin α5β1 can recognize and adhere to extracellular ligands containing RGD tripeptide motif. Research on the molecular interactions of integrin α5β1 may be essential for interpreting the biological function and underlying mechanisms of α5β1 integrin. Herein, we will discuss the reported ligands and related functions of integrin α5β1, which are summarized in Table 1. Extracellular matrix molecules fibrinogen, fibronectin, and fibrillin-1 could be recognized and bound by integrin α5β1, which have been shown to affect cell adhesion and migration of endothelial and other cells.[14,47–50] VEGFR-1 was secreted by endothelial cells and then interacted with integrin α5β1, and this interaction was important for angiogenesis.[51] CD97, CD87 and CD154, transmembrane proteins contain RGD peptide, have shown to interact with integrin α5β1 and induce cell

Table 1 Ligands and Related Functions of Integrin α5β1

Ligand	Functions	Reference
Fibronectin	Regulates cell adhesion and migration	[47]
Fibrinogen	Regulates cell adhesion and migration	[48]
Fibrillin	Regulates cell adhesion and migration	[50]
VEGFR1	Affects angiogenesis	[51]
CD97	Mediates migration and angiogenesis	[52]
CD154	Induces intracellular signaling	[53]
CD87 (uPAR)	Induces migration, invasion and angiogenesis	[54]
PHEV	Mediates actin cytoskeletal rearrangement	[55]
25-hydroxycholesterol	Regulates integrin signaling and cell adhesion	[56]
Tubulointerstitial nephritis antigen-like 1	Mediates FN-induced integrin/FAK signaling	[57]
Pregnancy-Specific Glycoprotein 1	Regulate extravillous trophoblasts migration	[58]
Neuropilin-2	Promotes cells extravasation and metastasis	[41]

adhesion, intracellular signaling, and angiogenesis.[52–54] Recently, other ligands of integrin α5β1 have been identified including Porcine hemagglutinating encephalomyelitis virus (PHEV), 25-hydroxycholesterol, Tubulointerstitial nephritis antigen-like 1 (Tinagl1), Pregnancy-Specific Glycoprotein 1 (PSG1), and Neuropilin-2.[41,55–58] All of these studies demonstrate that ligand-binding regulation of α5β1 integrin plays an important role for regulating the cellular function, such as cell adhesion, migration, and angiogenesis.

Functions of α5β1

As a transmembrane protein, integrin α5β1possess different domains include extracellular, transmembrane, and cytoplasmic domain that determines the multiple functions of α5β1. The extracellular and transmembrane domains are responsible for binding to ECM proteins, or other extracellular ligands, and contribute to subsequent signaling pathway function, whereas the cytoplasmic domain can interact with cytoskeleton-associated proteins to affect cell migration, invasion, and proliferation.[55,59–66] It also reported that integrin α5β1 was involved in anoikis resistance or drug resistance of cancer cells.[67–70] Besides, α5β1 integrin was strongly associated with cellular senescence.[71] Integrin α5β1 was also linked to the maintenance of bone tissue-forming and the formation of atherogenic inflammation, as well as the function/survival of T cell.[72–74] The multiple functions of α5β1 integrin indicated that dysregulation of α5β1 integrin could lead to various diseases, particularly cancer. Indeed, the hyperexpression of α5β1 integrin has been shown to promote tumor metastasis in lung cancer and melanoma. However, α5β1 integrin also act as a tumor-suppressive role in several breast cancer and colon cancer cell lines.[14]

Implication of α5β1 in Carcinogenesis

It is well known that integrin α5β1 acts an important role in diverse cancer progression and cancerogenesis, and thus the deregulation of α5β1 integrin is highly associated with a series of malignant tumors. Herein, we will discuss the potential roles and related functions of integrin α5β1 on human cancers in this section (Table 2).

Expression of Integrin α5β1 is Upregulated in Various Types of Cancers

Aberrant upregulation of integrin α5β1 has been implicated in a number of human malignancies and is closely correlated with poor prognosis. Integrin α5β1 expression was slightly expressed in normal brain tissue, but was expressed at significantly high intensity in glioblastoma tissue.[75] Research demonstrated that activation of α5β1 integrin was linked to the promotion of cell survival, migration, invasion, angiogenesis, and drug-resistance of glioma cells.[43,63,69,76,77] In addition, α5β1 integrin expression was overexpressed in colon cancer cells, and blockade of cell surface α5 integrin by selective antibody significantly suppressed cell adhesion and induced apoptosis.[78,79] In MCF-7 human breast carcinoma cells, hyperexpression of integrin α5β1 promoted cell invasion and doxorubicin resistance by enhancing the activity of AKT, mTOR, and ERK1/2 protein kinases.[68,80,81] Importantly, blocking α5β1 integrin by PHSCN (Pro-His-Ser-Arg-Asn) peptide significantly prevented cell metastasis in preclinical prostate adenocarcinoma models, and parallel progression

Table 2 Functional Roles of Integrin α5β1 Pathway in Different Types of Cancer

Cancer Type	Experimental Model	Function	References
Glioblastoma	Cell culture, Animal model	Promotes angiogenesis	[43]
Glioma	Cell culture	Drives migration	[63]
Glioblastoma	Cell culture	Increases resistance to temozolomide	[69]
Glioblastoma	Cell culture	Promotes cell proliferation	[76]
Glioblastoma	Cell culture	Resists apoptosis	[77]
Colorectal cancer	Cell culture	Promotes cell adhesion	[78]
Colorectal cancer	Cell culture	Promotes cancer resistance	[94]
Colon cancer	Cell culture	Regulates cell differentiation	[79]
Colon cancer	Cell culture, Animal model	Inhibits tumor metastasis	[108]
Colon cancer	Cell culture	Suppresses cell proliferation	[109]
Colon cancer	Cell culture	Inhibits cell apoptosis	[110]
Breast cancer	Cell culture	Promotes resistance to doxorubicin	[68]
Breast cancer	Cell culture	Regulates cell apoptosis and drug resistance	[80]
Breast cancer	Cell culture	Facilitates cell invasion	[81]
Prostate cancer	Cell culture, Animal model	Promotes tumor metastasis	[82]
Melanoma	Cell culture, animal model, clinical settings	Promotes tumor metastasis	[3]
Melanoma	Cell culture, animal model	Promotes tumor metastasis	[83]
Uveal melanoma	Cell culture	Inhibits tumorigenic properties	[114]
Lung cancer	Immunohistochemical analysis	Correlates with lymph node metastasis	[85]
Cervical cancer	Immunohistochemical analysis	Correlates with poor histologic differentiation and lymph node metastasis	[86]
Bulky squamous cervical cancer	Immunohistochemical analysis	Correlates with negative chemotherapy response and recurrence	[87]
Epithelial ovarian cancer	Cell culture, animal model	Inhibits tumor growth	[89]
Ewing sarcoma	Cell culture, animal model	Promotion tumor progression	[90]
Acute lymphoblastic leukemia	Cell culture	Facilitates cell adhesion and invasion	[91]
Basal Cell Carcinoma	Cell culture, animal model	Promotes cell invasion	[92]
Multiple myeloma	Cell culture	Facilitates cell adhesion and drug resistance	[93]
Osteosarcoma	Cell culture, animal model	Facilitates tumor metastasis	[95]
Squamous carcinoma	Immunohistochemical analysis	Promotes carcinogenesis	[96]
Head and neck squamous cell carcinoma	Cell culture	Increases EMT and metastasis	[97]
Mesothelioma	Cell culture, animal model	Promotes cell invasion	[98]
Pancreatic ductal adenocarcinoma	Cell culture, animal model	Promotes cell migration	[32]
Gastric cancer	Immunohistochemical analysis	Facilitates gastric carcinogenesis	[99]
Cholangiocarcinoma	Cell culture	Promotes cell invasion	[100]
Epidermoid carcinoma	Cell culture	Promotes cell proliferation	[101]
Epidermoid carcinoma	Cell culture, animal model	Facilitates tumor growth	[104]
Chondrosarcoma	Cell culture	Promotes cell motility	[102]
Neuroblastoma	Cell culture	Promotes cell motility	[103]
Motile carcinoma	Cell culture	Regulates cell adhesion	[105]
Rectal cancer	Immunohistochemical analysis	Functions as a predictive marker	[106]
Transitional carcinoma	Cell culture	Increases cell adhesion	[107]

Phase I clinical trial.[82] Besides, integrin α5β1 was upregulated in some primary and metastatic melanoma cells and positively linked to liver metastasis in melanoma.[3,83] In node-negative non-small cell lung cancer (NSCLC), the expression of α5β1 integrin was highly expressed in 50.0% (44/88) node-negative NSCLC patients and significantly associated with the differentiation status and age of the patients.[84] Notably, another study demonstrated that α5β1 integrin expression was more frequent in NSCLC with lymph node metastasis.[85] Integrin α5β1 was also overexpressed in 84.6% (143/169) cervical

cancer samples, and high α5β1 integrin expression was closely linked to poor histologic differentiation, lymph node metastasis, negative chemotherapy response, and recurrence in cervical cancer.[86,87] Researchers also showed that the α5 and β1integrin subunits were significantly increased in ovarian cancer compared with the normal tissue, and inhibition of the expression of integrin α5 and β1 may be improved the prognosis of ovarian cancer patients.[88,89] Patients with α5β1 integrin hyperexpression tended to have poor overall survival in ewing sarcoma, leukemia, basal cell carcinoma, multiple myeloma, colorectal cancer, osteosarcoma, squamous carcinoma, head and neck squamous cell carcinoma, mesothelioma, pancreatic carcinoma, gastric cancer, cholangiocarcinoma, epidermoid carcinoma, chondrosarcoma, neuroblastoma, epidermoid carcinoma, motile carcinoma, rectal cancer, and transitional carcinoma.[32,81,90–107]

Integrin α5β1 as a Tumor Suppressor in Several Types of Cancer Cell Lines

Integrin α5β1 as a classic cell surface receptor has been reported as a tumor suppressor due to overexpressing α5β1 integrin in tumor cells are less tumorigenic than its corresponding parent cells. In colon cancer cell line, HT29, α5β1 integrin overexpression showed a strong inhibitory function on lung colonization and metastasis.[108] And de novo expression of α5 integrin subunit was linked to suppress cell growth arrest and retard the tumorigenic growth of HT29 cells.[109] However, another study demonstrated that upregulation of the α5 integrin subunit suppressed apoptosis triggered by serum deprivation in HT29 cells.[110] Then, further research demonstrated that integrin α5β1 level was significantly elevated in the poorly differentiated colon cancer cell lines and was positively associated the tumorigenic capacity. Therefore, the different roles of α5β1 integrin in colon cancer cells might be related to the differentiation status.[111] Besides, loss of α5β1 integrin at the cell surface of the uveal melanoma cells was positively associated with the high tumorigenicity and aggressiveness.[112–114]

Deregulation of Integrin α5β1 Exerts Dramatic Effects on Diverse Cellular Functions

Role of Integrin α5β1 in Angiogenesis

Angiogenesis is a crucial physiological and pathological process for the development of new blood vessels, which was responsible for the tissue repair and fertility, embryonic development, chronic inflammation, tumor growth and metastasis.[115] Basic and clinical studies demonstrated that inhibition of angiogenesis could suppress tumor metastasis and progression. Most studies implicate integrins, which are critical modulators of tumor angiogenesis. Among the integrin family, αv, α2, α3, α4, α5, β1 or β2 integrin subunits have been demonstrated to be associated with vasculo- and angiogenesis during development.[5,14,65] Research found that loss of fibronectin, the major ligand for α5β1 integrin, led to angiogenesis abnormalities and embryonic death at E9.5 in mice.[116] Genetic ablation studies have indicated that β1-integrin-null endothelial cells displayed vascular remodeling effects resulting from adhesion and migration alteration, suggesting the β1 integrin family clearly played an important role in angiogenesis.[14,117] Integrins α5 and αv have been considered as key modulators of endothelial cells and vascular smooth muscle cell (vSMC) function. Interestingly, endothelial cell-specific knockout of either integrin α5 and αv do not have obvious angiogenesis defects during embryonic development.[118] Researchers found that vSMC-specific knockout of both α5 and αv integrin led to the formation of large aneurysms within the brachiocephalic/carotid arteries and cardiovascular defects, as well as late embryonic lethality.[119] These studies indicate that specific integrins are important during the vascular development, and the compensation mechanisms by other integrins are essential for normal angiogenesis.

In most quiescent endothelium, integrin α5β1 was limited to very low levels, but its expression was significantly upregulated in tumor vasculature or neovessels.[120,121] Integrin α5β1 participated in regulating angiogenesis by interacting with diverse partners such as CD97, angiopoietin-2 (Ang-2), CD87, VEGFR1, and endostatin.[51,54,122–124] Integrin α5β1levels in endothelial cells were induced in response to several angiogenic factor stimuli, such as IL-8, bFGF, EGFL7, Del-1or TNFα, but not by VEGF.[43,120,125] Besides, HoxD3 acts as a homeobox gene controlled the expression of integrin α5 by directly binding to the promoter of the α5 subunits.[126] Therefore, integrin α5β1 plays an important role in angiogenesis, and blocking α5 and β1 integrin subunits by specific monoclonal antibodies or small peptides has become a potential strategy for anti-angiogenesis therapy.[127–130]

Integrin α5β1 Regulates the Migration and Invasion of Tumor Cells

The ability of cancer cells to invade locally and further form distant metastasis is partly determined by integrin-mediated attachment to ECM. Integrin α5β1 function as a critical

regulator for tumor cell migration and invasion by affecting cytoskeleton rearrangement, cell adhesion, and the production of matrix metalloproteinase (MMP). Some studies have demonstrated that integrin α5β1 enhanced keratinocyte adhesion to fibronectin, and promoted invasion and metastasis via activating various signaling pathways.[11,131] Besides, fibronectin binding to integrin α5β1 led to the direct association of α5 integrin with c-Met, which was upstream of Src and FAK. Integrin α5β1 promoted tumor cells invasion and metastasis via activating the c-Met/FAK/Src-dependent signaling pathway.[132] Research also found that integrin α5β1 promoted invasiveness and metastasis by regulating the expression and/or activity of MMPs.[133] In breast cancer, cells with high integrin α5β1 expression elevated a 3-fold invasive capacity compared with cells exhibiting low α5β1 levels.[81] Integrin α5β1 could direct recruit MMP2 collagenase on the surface of breast carcinoma cells, and then regulating cell invasion by control of the levels of MMP2.[134] Moreover, MMP-2/α5β1 binding has pivotal role in regulating tumor metastasis by inducing α5β1-mediated IL-6/STAT3 signaling pathway.[135] In murine cell line B16F10, integrin α5β1and fibronectin interaction facilitated cell invasion by inducing the activity, mRNA, and protein expression of MMP9. Blocking the α5 integrin receptor by specific antibody remarkably abrogated the fibronectin-induced MMP9 response.[136] Moreover, ADAM Metallopeptidase Domain 17 (ADAM17) was reported to directly interacted with integrin α5β1, and this interaction might take place on the same cell or on different cell, with the function to affect cell-cell adhesion and migration.[137,138] In addition, Rab-coupling protein (RCP)-driven endocytic recycling of α5β1 integrin also promoted invasion of cancer cells, which was associated with actin cytoskeleton arrangement.[7] The effects of integrin α5β1 on the cell adhesion, migration, and invasion of tumor cells indicate that it may be used as the biomarker for the metastasis of tumors.

Integrin α5β1 Mediates the Proliferation of Tumor Cells

Mounting studies have implicated that integrin α5β1 contributed to tumor cell proliferation in vitro and tumor growth in vivo. It has been demonstrated that α5β1 integrin possessed the ability of enhancing cell proliferation depending on the fibroblasts, which are some of the major cells in neoplasm tissues and affect cancer progression. High L1 cell adhesion molecule (L1CAM) levels in fibroblasts promoted cancer cell proliferation by targeting integrin α5β1.[139] In addition, interaction of myofibroblasts and soluble fibronectin facilitated the α5β1 integrin-dependent tumor growth in the hepatocellular carcinoma.[140]

Integrin α5β1 was also considered to be an important regulator for cell cycle-associated proteins. p53 is a universal tumor suppressor implicated in cell cycle arrest, apoptosis, and DNA repair. Research found that the expression of α5 integrin subunit was negatively associated with p53 activity, and depletion of the α5 integrin subunit could increase p53 activity.[69] Interestingly, re-activation of p53 by Nutlin-3, a p53-reactivating compound, significantly inhibited the mRNA and protein expression of α5 integrin subunit.[141] Thus, the crosstalk between α5β1 integrin and p53 was crucial for tumor growth, and some antagonists of α5β1 integrin have been applied by modulating the integrin α5β1/p53 pathway.[77,142] Of note, the integrin α5β1-ERK pathway was also involved in the regulation of cancer cell proliferation.[104] Antibodies that block the integrin α5β1 negated the proliferative effect of integrin α5β1 in malignancy cells.[143]

Role of Integrin α5β1 in Chemoresistance and Radioresistance

Resistance to chemotherapy and radiotherapy is a unique hallmark of neoplasm and is responsible for tumor recurrence and patient relapse.[144] Cell adhesion to ECM components is a critical determinant of chemotherapeutic response of human cancers, such as myeloma. For example, Integrin α5β1 promoted K562 chronic myelogenous leukemia (CML) cells bind to fibronectin, and this binding was resistant to apoptosis induced by chemotherapeutic drugs and γ-irradiation.[145] Besides, fibronectin/integrin α5β1 binding elevated the efficiency of 2-D colony formation, and provided resistance to paclitaxel-mediated apoptosis.[146] Research has demonstrated that integrin α5β1 protected high-grade glioma cells from temozolomide-induced apoptosis by interfering with the p53 pathway in glioma.[69] In epithelial ovarian carcinomas, overexpression of integrin α5 was a strong risk factor for drugs resistance.[147] Integrin α5β1 also contributed to cell adhesion and drug resistance of multiple myeloma cells through activating the FAK/STAT3/AKT pathways.[93] In MCF-7 human breast carcinoma cells, hyperexpression of integrin α5β1 promoted the doxorubicin resistance in an ERK-dependent manner. Besides, silencing of integrin α5β1 significantly inhibited the activity of kinases AKT and ERK in MCF-7 doxorubicin-resistant cells.[80]

The Signaling Pathways Involved in Integrin α5β1-Mediated Tumor Progression

Integrin α5β1 functions as a cell surface receptor, and ligation of integrin α5β1activates several crucial signaling pathways that are critical in carcinogenesis/tumor progression, such as FAK signaling, Wnt/β-catenin signaling, NF-κB signaling, Yes-associated protein (YAP) signaling, and ERK signaling. Interpreting the molecular mechanism of integrin α5β1 in these pathways may provide a better understanding of carcinogenesis/tumor progression.

Regulation FAK Pathway by Integrin α5β1

Due to lack of intrinsic tyrosine kinase activity, integrins transduce extracellular cues to intracellular signaling pathways require non-receptor tyrosine kinases such as FAK.[148,149] Research demonstrated that fibronectin-integrin α5β1 complex facilitated the auto-phosphorylation of the Tyr 379 residue on FAK. Subsequently, the tyrosine kinase Src bound to phosphorylated FAK through its SH2 domain, and induced phosphorylation of FAK at Tyr 925 residue, which then promoted the formation of FAK-Grb2-SOS complex. Ultimately, this complex contributed to cell proliferation, metastasis, and tumorigenesis of cancer cells by activating Ras GTPase and inducing the MAPK/ERK signaling pathway.[150,151] Indeed, integrin α5β1-FAK signaling pathway contributed to cell metastasis and cancer progression of several malignant neoplasms, and some specific monoclonal antibody or integrin α5β1 inhibitor could significantly negate these accelerative effects.[57,152–154]

Regulation Wnt/β-Catenin Pathway

Wnt/β-catenin pathway is critical to facilitate tumor progression, such as cell proliferation, cell cycle, cell metastasis, differentiation, and apoptosis. In pancreatic cancer, integrin α5β1 mediated the adhesion of pancreatic adenocarcinoma cells on fibronectin under serum-free conditions, resulting in the increasing of β-catenin localization throughout the cell.[155] In hepatocellular carcinoma, overexpression of CD147 competitively bound to integrin β1that interrupted the fibronectin/integrin β1interaction, which is responsible for E-cadherin degradation and β-catenin nuclear translocation.[156] In glioma cells, overexpression and activation of α5β1 integrin by fibronectin facilitated the transactivation of β-catenin gene targets and induced an increase in cell migration.[63] In addition, other high-affinity peptide such as cyclized CRRETAWAC also promoted integrin α5β1-mediated Wnt/β-catenin transcriptional activity.[157]

Regulation of NF-κB Signaling

NF-κB transcription factors and their regulated genes have been recognized as critical mediators involved in tumor initiation, cell proliferation, survival, metastasis, angiogenesis, and resistance to chemotherapy and radiotherapy.[158] Fibronectin/integrin α5β1 interaction was responsible for inducing the expression of the p65 component of NF-κB and enhancing the DNA-binding activity of NF-κB in human bronchial epithelial cells.[159] Upon fibrinogen binding, integrin α5β1 and αvβ3 promoted the activation of NF-κB and increased the expression of NF-κB-mediated inflammatory chemokines in endothelial cells. And these effects were inhibited by blockage of the integrin α5β1 and αvβ3 with the GRGDS peptide.[160] Besides, lunasin, a naturally occurring 43-amino acid peptide isolated from soybean, direct binding with integrin α5β1 and inhibiting the NF-κB signaling in colon cancer cells.[152]

Activation of YAP by Integrin α5β1

YAP, the crucial transcriptional regulator of the Hippo pathway, is involved in modulating organ size, tissue homeostasis and repair, and tumorigenesis.[161] Hyperactivation of YAP is associated with the malignant behavior of neoplasm, such as high proliferation, invasion into the surrounding normal tissue, vascularization, and drug resistance.[162] Recent studies demonstrated that activation of integrin α5β1 by the ligand fibronectin significantly increased the phosphorylation of YAP at Tyr357 and induced YAP nuclear translocation via the tyrosine kinase c-Abl in ECs. In contrast, blockage of integrin α5β1 with ATN161or inhibition of c-Abl with bosutinib markedly reduced the levels of integrin α5β1 and p-YAP^{Y357}.[163] Beyond YAP phosphorylation, the dephosphorylation of YAP (S127) was also regulated by the α5 integrin subunits.[164] In Ewing sarcoma cells, integrin α5β1signaling was associated with YAP dephosphorylation and nuclear translocation, and this signaling pathway significantly promoted tumor progression.[90]

Integrin α5β1 Regulates ERK Signaling Pathway

ERK signaling pathway is hyperactivated in a variety of cancers, which execute programmes related to cell cycle, differentiation, migration and invasion, and apoptosis. Fibronectin and integrin α5β1 binding enhanced Hela cell proliferation by increasing the phosphorylation of ERK at Thr 202 and Tyr 204 residues and then activating the ERK pathway.[148] uPAR (CD87), an urokinase receptor frequently upregulated in several types of tumors, bound to integrin α5β1 and then persistently activated the ERK

signaling.[165] Using site-directed mutagenesis, two single amino acid mutants of the uPAR (S245A and H249A) were respectively identified that fail to facilitate integrin α5β1-mediated ERK signaling.[104,166] Moreover, disruption of uPAR/integrin α5β1 interaction by using specific small molecules significantly inhibited ERK activity and tumor progression.[104,167]

Translational Implications of Integrin α5β1 in Cancer

Integrin α5β1 as a Target for Imaging

Although integrin α5β1 is limited to very low levels in quiescent endothelial cells, it is significantly upregulated in tumor vasculature or neovessels.[120,121] And α5β1 integrin is strongly correlated with tumor angiogenesis, suggesting it may be a potential predictive target. So far, several imaging probes for α5β1have been described for tumor molecular imaging. Stefanie et al firstly developed the α5β1-selective antagonists labeled with $^{68}Ga^{3+}$ for PET (positron emission tomography) imaging and could verify different patterns of integrin α5β1expression in tumors.[168] D'Alessandria et al then successfully developed a ^{68}Ga-labelled α5β1-selective peptidomimetic named FR366, which showed good image quality for PET imaging.[169] Through sequential N-methylation analysis, Tobias et al discovered a most potent and selective α5β1-integrin ligand peptide, c(phg-isoDGR-(NMe)k), which was applied for PET imaging by trimerized with the chelator TRAP and labeled with ^{68}Ga.[170] In addition, there have been several α5β1-specific probes such as ^{99}m Tc-HisoDGR, ^{99}mTc-AB -3PisoDGR2, and ^{99}mTc-3PisoDGR were developed for SPECT (single-photon emission computed tomography) imaging.[171,172] Recently, RNA aptamers have received attention as promising tools for clinical applications due to their smaller size, lack of immunogenicity and toxicity, temperature stability, ease of chemical modification, and lower cost of production.[173,174] Fechter and colleagues successfully identified and developed RNA aptamers, aptamer H02, is efficient to distinguish GBM tumor tissues from patient-derived tumor xenografts. This new, original, and powerful aptamer tool may be open roads for α5β1-specific clinical therapy.[175]

Integrin α5β1 as a Target for Therapy

Integrin α5β1 has become a potential target for cancer therapy, and several specific α5β1 integrin antagonists have been developed and used in preclinical or clinical studies. These antagonists are mainly presented as anti-angiogenic agents due to the pro-angiogenic function of integrin α5β1, and they mainly consisted by specific antibodies and small peptides.

A series of blocking antibodies was developed to target the interaction between integrin α5β1 and fibronectin. IIA1, an integrin α5β1 function-blocking murine antibody, was generated and used to inhibit in vitro angiogenesis, cell adhesion, invasion, and survival of tumor cells.[46,130,176] Notably, Ramakrishnan and colleagues firstly developed a chimeric human IgG4 version of IIA1 antibody, volociximab, with similar affinity for α5β1 integrin and similar activity by inhibition of fibronectin binding than IIA1.[130] Volociximab as a potential anti-antigenic drug and has been shown to be effective, safe, and tolerable in phase I b studies in patients with non-small-cell lung cancer, and in Phase II studies in patients with epithelial ovarian or primary peritoneal cancer.[177–179] The MINT1526A is a function-blocking anti-α5β1 monoclonal antibody, has been used in anti-angiogenic therapy combining α5β1 and VEGF inhibition, and has been shown to be well tolerated and safe in phase I study.[180] Recently, a bispecific antibody (BsAbα5β1/αv) simultaneously targeting the degradation of αv and α5β1 integrins. And this combinatorial strategy was superior to monospecific antibodies in abrogating cell adhesion, migration, survival in prostate cancer cells.[181]

Integrins α5β1can recognize the RGD motif of fibronectin and then directly bind to it. Recent years, many studies focus on the design antagonists with enhanced selectivity of α5β1 integrin. Some antagonists have been developed and used in preclinical or clinical studies, such as SJ749 and JSM6427, and ATN-161.[120,142,182,183] Among them, ATN-161 (Ac-PHSCN-NH2), a competitive inhibitor of the FN-α5β1 interactions firstly developed by Attenuon LLC (San Diego, CA, USA), have moved to Phase II clinical trails.[14]

Although many preclinical studies supported the anti-angiogenic therapies of blocking antibody and small molecule, early clinical responses have been disappointing. Most α5β1 integrin inhibitors and antibodies were clinical investigations in phases 1 or 2 that have not progressed through phases 3, revealed no treatment benefit.[184] Murphy and colleagues revealed that FN and the FN receptors, α5 and αv, were dispensable for tumor angiogenesis through a series of genetic tools and pre-clinical models (transplant models and *RIP1-Tag2* model of pancreatic cancer), suggesting that the antagonism of antibodies or small molecules on tumor angiogenesis may occur through a dominant-negative effect, rather than a simple

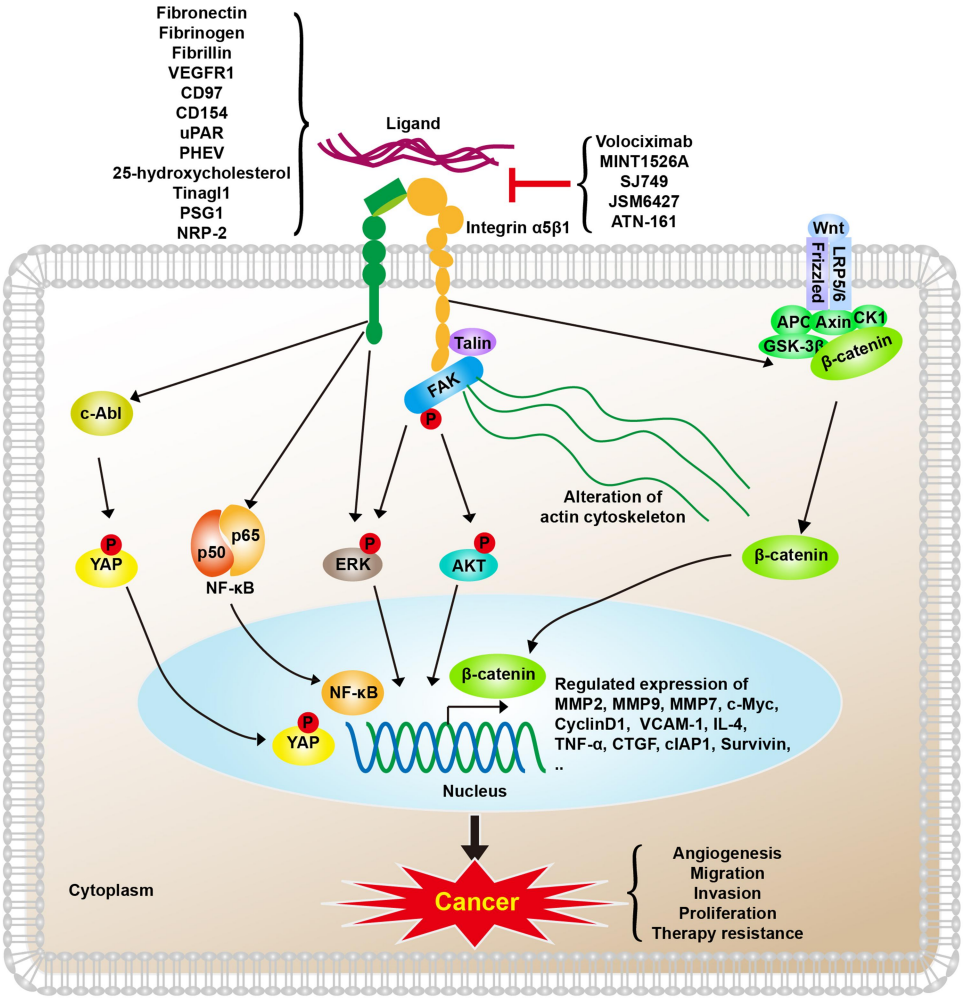

Figure 1 A schematic model by which integrin α5β1/ligand binding contributes to cancer progression through regulating several crucial signaling pathways such as FAK signaling, Wnt/β-catenin signaling, NF-κB signaling, YAP signaling, and ERK signaling.

block of the FN-integrin α5β1binding.[184] And they also found that tumor growth was not affected by the absence of FN and its integrin receptors.[184] In addition, they revealed the potential compensatory mechanism that several RGD-containing extracellular matrix proteins, such as fibrillins, collagens, and nidogens, might be important in compensating for the loss of FN.[184] Therefore, further in vivo genetic studies were necessary to resolve the targeting difficulties.

Conclusion

In this review, we briefly illustrate our understanding on the structure, regulation, ligands and biological functions of integrin α5β1, and reveal the roles of integrin α5β1in various tumors. The dysregulation of α5β1 integrin significantly relates to the development and progression of many neoplasms and can be used as a valuable indicator of poor prognosis. Functionally, integrin α5β1 can recognize and adhere to extracellular ligands containing RGD tripeptide motif, and this integrin α5β1/ligand binding modulates diverse cellular progression by activation of several classic oncogenic signaling pathways, such as FAK signaling, Wnt/β-catenin signaling, NF-κB signaling, YAP signaling, and ERK signaling (Figure 1). The important role of integrin α5β1 in the tumor angiogenesis is that provides the potential predictive possibility for tumor molecular imaging, such as PET, SPECT, and RNA aptamers. Moreover, several specific α5β1 integrin antagonists have been developed and/or used in preclinical or clinical studies. Considering the pivotal cellular role of the integrin α5β1, it is reasonable to assume that advances in integrin α5β1 research will facilitate the development of molecular diagnosing and therapy of tumors in the future.

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Author Contributions

All authors made substantial contributions to conception and design; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest.

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