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Integrin β4 in Breast Cancer: A Focused Review

Zoya Salahuddin[1], Shuhan Ying[2], Emily Xie[3], Aizhang Xu[4], Xiaojing Ma[4*]

[1]Weill Cornell Medicine-Qatar, Doha, Qatar
[2]Syosset High School, Syosset, New York
[3]Department of Mathematics, University of California, San Diego, USA
[4]Department of Microbiology and Immunology, Meyer Cancer Center, Weill Cornell Medicine, New York, USA
Email: *xim2002@med.cornell.edu

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Abstract

Integrin β4 (ITGB4) is emerging as a pivotal player in breast cancer progression, particularly in aggressive and invasive subtypes such as triple-negative breast cancer (TNBC). This review broadly summarizes current research on ITGB4's involvement in breast cancer, focusing on its role in metastasis, metabolic reprogramming, drug resistance, and immunoregulation that collectively influence tumor behavior with implications for the development of innovative and more efficacious therapeutic modalities.

Keywords

Integrins, ITGB4, Prognosis, EMT, Angiogenesis, Drug Resistance, Cancer Therapy

1. Introduction

Breast cancer continues to pose a significant challenge to science and healthcare, despite substantial advances in treatment and management modalities [1]. The disease remains a leading cause of cancer-related deaths among women, necessitating deeper insight into mechanisms of cancer progression and resistance to therapy, a domain where integrins prove to play a key role.

Integrins are a superfamily of transmembrane cellular adhesion molecules, aptly named for their role in integrating signals from the extracellular environment into the cell. Structurally, they consist of paired alpha (α) and beta (β) units that bind to extracellular matrix ligands, cell-surface ligands, and soluble ligands. In mammals including humans and mice, 18 α and 8 β subunits combine to form 24 functional integrin heterodimers [2]. In particular, the α6 integrin subunit is a recognized stemness marker, prominently expressed on the plasma membrane of stem cell populations. It forms heterodimers with either the β1 or β4 (ITGB4)

integrin subunits [3].

Integrin β4 (ITGB4), also known as CD104, is encoded by the name-sake gene located on human chromosome 17 as a protein of 1822 amino acids, whereas the murine counterpart is located on chromosome 11 encoding a protein of 1805 amino acids that shares ~88% identity with the human orthologue. Low to moderate levels of ITGB4 protein are found in most normal human tissues, except in the placenta where ITGB4 is abundant (**Figure 1(A)**). At the single cell mRNA level, salivary duct cells appear to express copies of amounts of ITGB4 (**Figure 1(B)**). This knowledge is relevant to understanding the general and specific function of ITGB4 in various tissues and cell types.

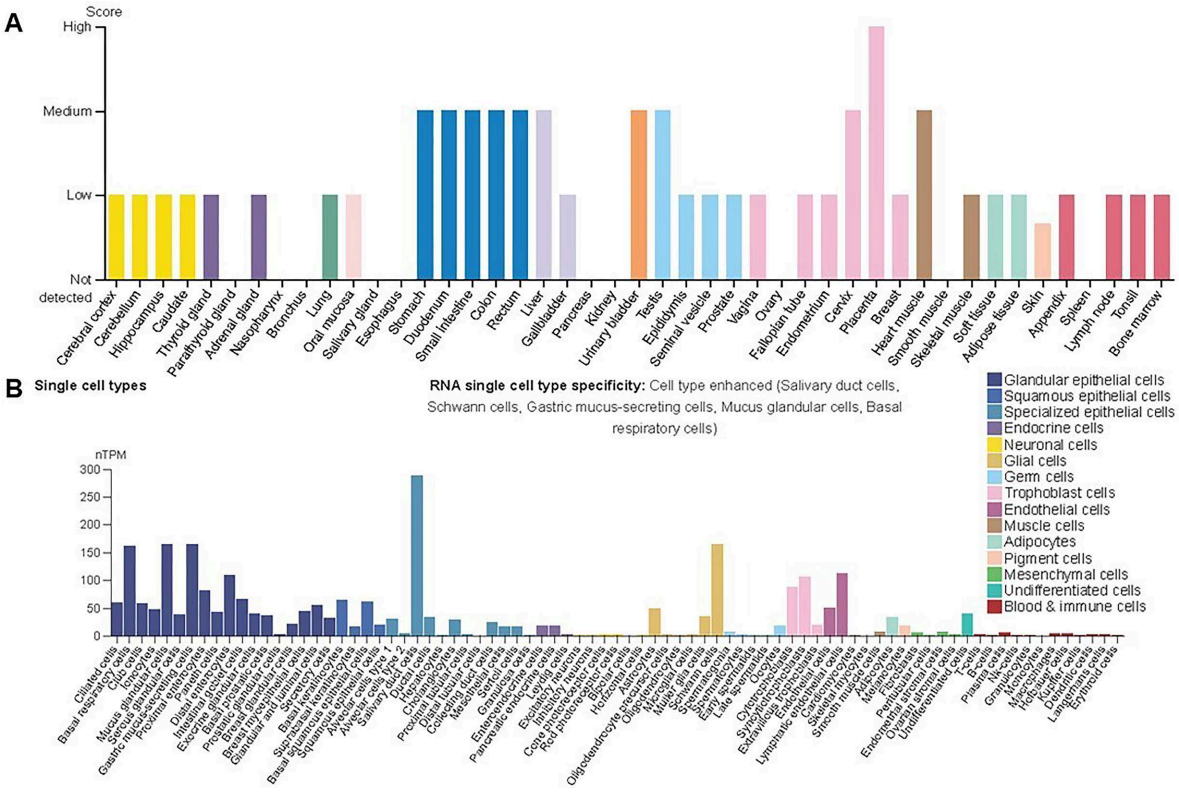

Figure 1. (A) ITGB4 protein expression data is shown for each of the 44 tissues based on knowledge-based annotation. (B) A summary of normalized single cell RNA (nTPM) from all single cell types. Color-coding is based on tissue groups, each consisting of tissues with functional features in common. Data source: The Human Protein Atlas.

The atypical intracellular domain of the β4 subunit is distinct in both size (~1000 amino acids) and structure from any other integrin subunit [3]. This domain functions as a key component of hemidesmosomes, and as a receptor for laminin, anchoring epithelial cells to the basement membrane. In the breast tissue, this anchorage not only provides structural stability and facilitates cell adhesion, but also contributes to mammary gland development. It does this by sustaining parathyroid hormone-related protein (PTHrP) signaling in breast epithelium, thereby aiding in growth and branching of breast tissue [3]. ITGB4 expression in mammary alveolar progenitor cells is also crucial in alveologenesis and milk

production during pregnancy [4].

The $\alpha 6\beta 4$ integrin heterodimer can interact with various extracellular matrix (ECM) molecules to activate intracytoplasmic mediators, allowing it to play a role in various cell survival pathways by altering the actin cytoskeleton and gene expression [5]. ITGB4 has garnered significant attention due to its overexpression in aggressive cancer phenotypes, particularly in breast cancer, where it is associated with poor prognosis, by facilitating the migration, invasion, and survival of carcinoma cells [6]. This review delves into the multifaceted roles of the integrin $\beta 4$ (ITGB4) subunit in breast cancer, exploring recent findings that highlight its contributions to tumor progression and its emerging potential as a target for therapeutic intervention [7] by systematically analyzing more than 120 research articles published to date via PubMed as well as several specific public databases for gene expression information.

2. ITGB4 Expression in Breast Cancer

The overexpression of ITGB4 in breast cancer is well established [7]. High ITGB4 mRNA expression in biopsy samples at diagnosis is strongly associated with lower relapse-free survival in TNBC patients treated with chemotherapy [8]. This suggests that ITGB4, which is expressed in mesenchymal TNBC cells, could be a valuable prognostic biomarker for high-risk TNBC. Interestingly, studies have found its expression in transitional, but not non-invasive, breast cancer cells [9]. The transcriptional regulation of ITGB4 in breast cancer has not been documented. C-Myc, KLF4 and ZNF304 have been described as transcriptional activators of ITGB4 in colorectal carcinoma, glioma, and ovarian cancer, respectively [10]-[12]. On the other hand, p53 was reported to inhibit ITGB4 transcription in conjunction with homeodomain interacting kinase 2 in breast cancer cell lines [13].

Huang *et al*. conducted a comprehensive pan-cancer analysis of ITGB4, revealing its oncogenic roles across various cancers, including breast cancer. Their findings demonstrated a novel, statistically significant positive correlation between ITGB4 expression and the immune infiltration level of cancer-associated fibroblasts in several tumors [14]. Additionally, through immunohistochemical staining, Li *et al*. reported that ITGB4 is an effective marker for lymphatic and blood vascular invasion, as well as perineural aggression in various malignancies including breast cancer [15].

A further analysis of The Cancer Genome Atlas (TCGA) data involving large cohorts of patient samples reveals that ITGB4 is amplified in breast cancer, particularly in the metastatic and invasive types (**Figure 2(A)**). These genomic alterations are strongly associated with poor relapse-free survival of patients with drastically shortened median survival time from 241 months to 86 months (**Figure 2(B)**), whereas no significant difference in disease-free survival was observed ($p = 0.0727$) (not shown), suggesting that the treatments that these patients had received may be effective in preventing relapse, but may not be as effective in

preventing new, separate cancers from developing in the same or different organs. A more granular view of the data confirmed that gene amplifications of ITGB4 in tumor tissues correlate well with increased expression (Figure 2(C)).

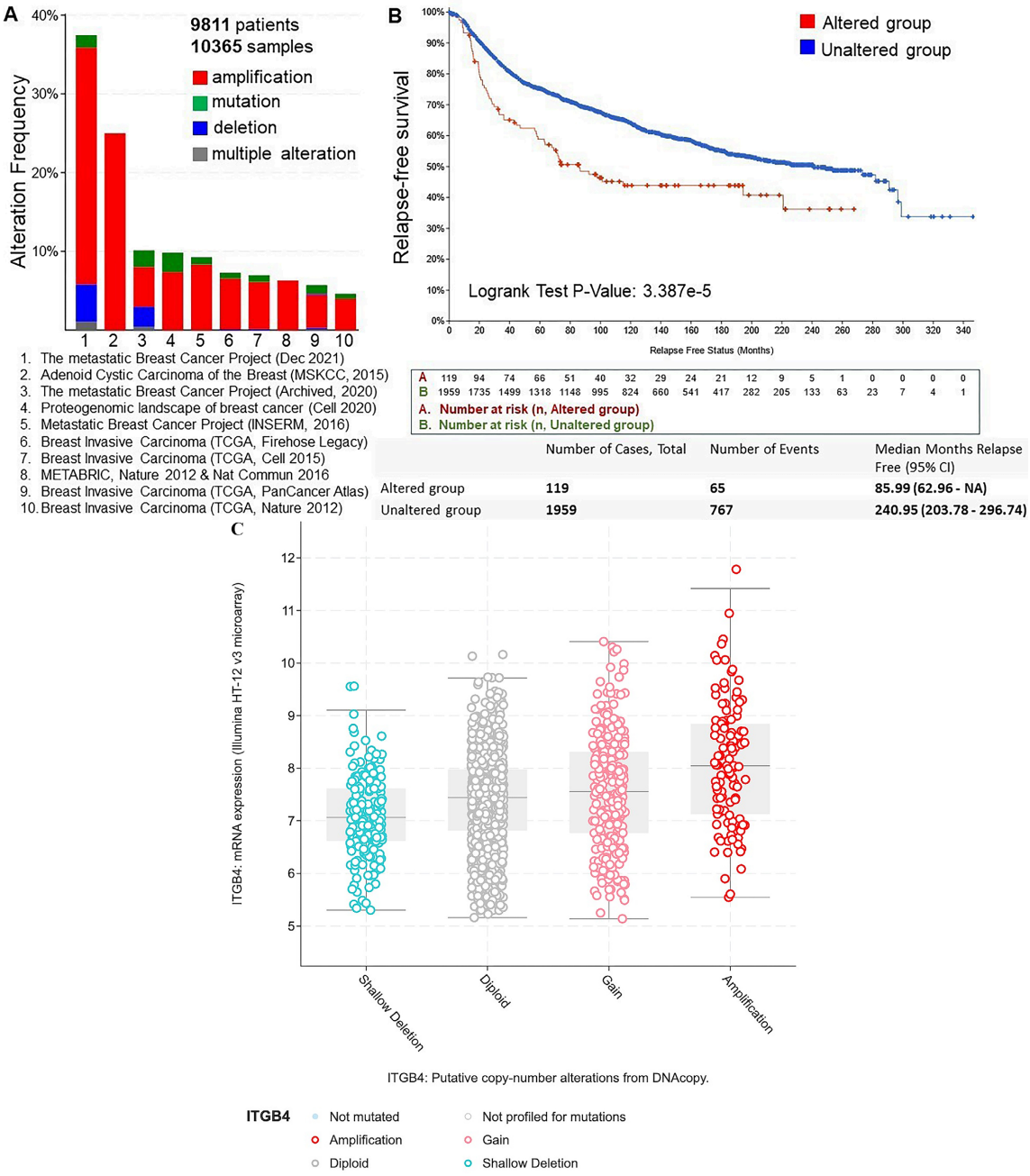

Figure 2. Genomic amplifications of ITGB4 in invasive breast cancer. Data in cBioPortal were analyzed for gene alteration frequencies in ITGB4 (A), their association with relapse-free survival (B). The data are derived from 10 studies involving 10,365 samples from 9811 patients. For the prognosis analyses, a total of 8,481 non-overlapping patient samples, divided into "Altered group" (627 samples) and "Unaltered group" (7854 samples), were analyzed. No statistical significance was found for disease-free, disease-specific, progression-free, and overall-survivals. (C) ITGB4 mRNA expression in tumors is correlated with different mutational status. Data source: https://doi.org/10.1038/ncomms11479. In this study, 173 genes were sequenced in 2433 primary breast tumors that have copy number aberration (CNA), gene expression and long-term clinical follow-up data.

Moreover, single cell RNA-sequencing analyses of human breast cancer tissues reveal that within the tumor microenvironment (TME), there are several cell types besides the epithelial cancer cells, *i.e.*, B cells, cancer associated fibroblasts (CAFs), endothelial cells, myeloid cells, plasmablasts, perivascular like (PVL) cells, and T cells (**Figure 3(A)**), which express ITGB4 to varying degrees (**Figure 3(B)**). However, endothelial cells appear to be a major ITGB4-expressing cell type (**Figure 3(C)**, **Figure 3(D)**), suggesting a significant role of ITGB4 in angiogenesis within the TME. This finding supports the notion that ITGB4 may play distinct roles in both the endothelial compartment, related to vascularization of the tumor, and in epithelial cancer cells, likely contributing to cancer progression.

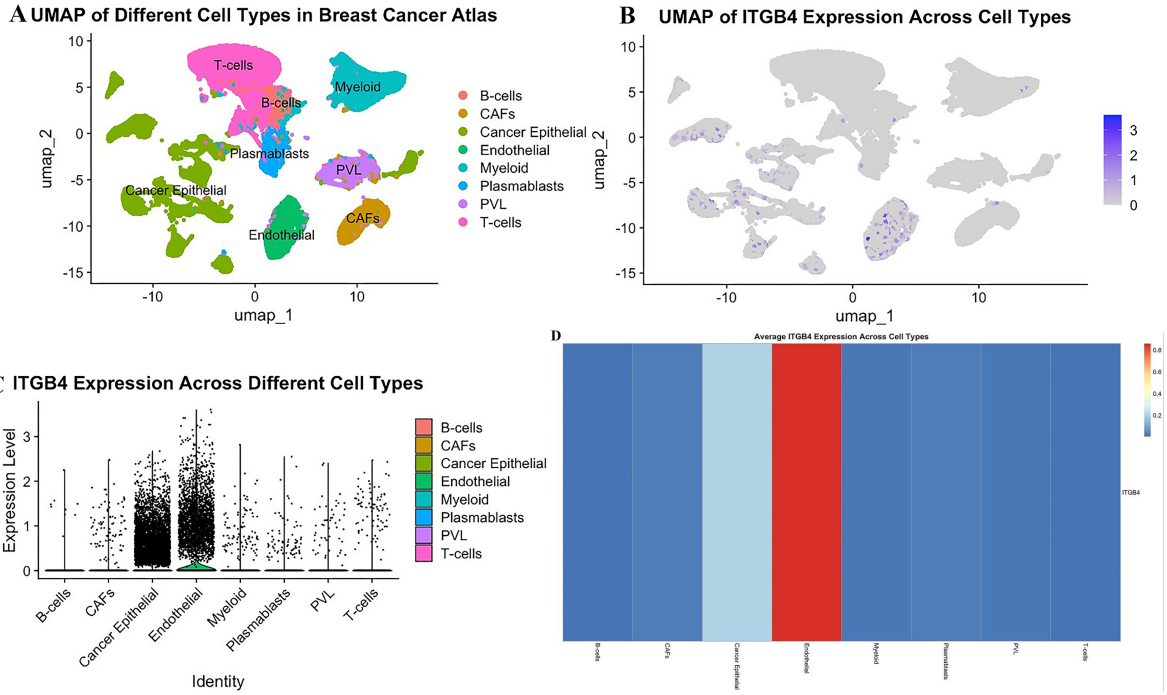

Figure 3. ITGB4 expression in the tumor microenvironment. (A) UMAP of Different Cell Types in Breast Cancer Atlas. This UMAP plot represents the clustering of different cell types from a single-cell and spatially resolved atlas of human breast cancers. The cell types shown include B-cells, CAFs, Cancer Epithelial cells, Endothelial cells, Myeloid cells, Plasmablasts, PVL, and T-cells. Each cluster is labeled and color-coded according to the respective cell type. The plot illustrates distinct clustering of each cell type based on their transcriptomic profiles, providing an overview of cellular heterogeneity within the TME. (B) UMAP of ITGB4 Expression Across Cell Types. This UMAP plot shows the expression of ITGB4 across the different cell type clusters displayed in A. The color gradient from gray to blue indicates increasing levels of ITGB4 expression. (C) ITGB4 Expression Across Different Cell Types. This violin plot displays the distribution of ITGB4 expression levels across various cell types. The Endothelial cells exhibit the highest concentration and levels of ITGB4 expression. Cancer Epithelial cells show the second-highest expression of ITGB4, although with a broader and less dense distribution. (D) Heatmap of Average ITGB4 Expression Across Cell Types. This heatmap shows the average expression levels of ITGB4 across different cell types.

Brendle *et al*. identified polymorphisms in predicted microRNA-binding sites within integrin genes, including ITGB4, as potential prognostic markers for breast cancer. They found that the A allele of the ITGB4 SNP rs743554 was associated

with a negative hormone receptor status, and poor overall survival. They hypothesized that the variant allele could cause a loss of the miRNA miR-34a binding site, a microRNA involved in suppression. This loss may enhance the ability of ITGB4 to promote tumor cell growth, invasion and survival [16]. The work signifies the importance of genetic variations in ITGB4 that could influence cancer prognosis and therapy responsiveness, highlighting patients with these specific polymorphisms as candidates for integrin-targeted therapies.

Together, these studies solidify the notion that ITGB4 expression could serve as a valuable biomarker for assessing the aggressiveness of breast cancer and guiding treatment decisions.

3. Mechanism of ITGB4 in Breast Cancer Progression

ITGB4 stands out among integrin subunits due to its unique and extended cytoplasmic tail, composed of distinct sequences not found in other b integrins, *i.e.*, a Calx b motif adjacent to the plasma membrane, two pairs of fibronectin type III (FnIII) domains, a connecting segment, and a COOH-terminal end domain, which enable ITGB4 to perform a number of cellular activities (**Figure 4**). In hemidesmosomes, the β4 subunit mediates many of the intracellular connections of the ITGB4 receptor, including those with cytoskeletal keratins via plakins, such as plectin. Both subunits bind to extracellular laminins, particularly laminin-332 [17].

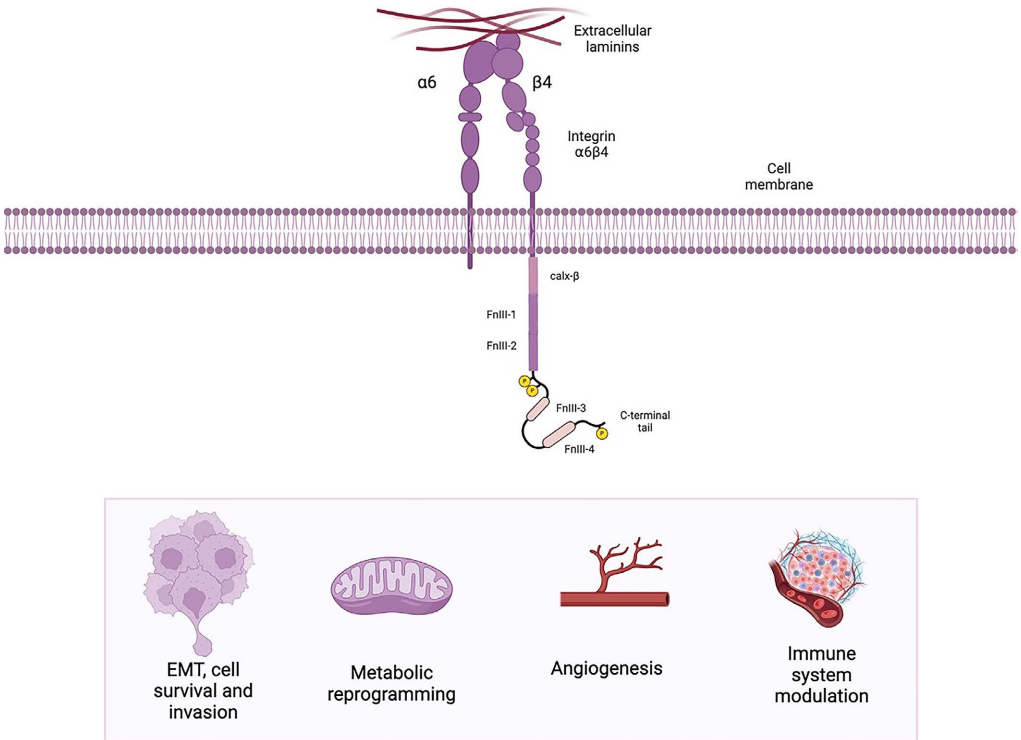

Figure 4. ITGB4-mediated pathways in breast cancer progression and therapy. With its long intracellular domain, ITGB4 has the potential to mediate various pathways involved in tumor progression, including metastasis, metabolic reprogramming, angiogenesis, and immune system modulation. Figure created on BioRender.

The dissolution of hemidesmosomes is a regular and necessary process for cellular dynamics, triggered by the phosphorylation of serine residues on the ITGB4 C-terminal tail in response to growth factors such as epidermal growth factor (EGF) [18]. This disassembly allows ITGB4 to orchestrate various aspects of tumor progression, including sustaining proliferative signaling, tumor invasion and metastasis, evasion of apoptosis, and stimulation of angiogenesis [19].

3.1. Invasion and Metastasis

Malignancy progression is dependent on the complex interactions of tumor cells with their microenvironment, followed by their dissemination through blood or lymphovasculature, known as metastasis [20]. A critical initial step in this metastatic cascade is the epithelial-to-mesenchymal transition (EMT), a biological process endowing epithelial cells with mesenchymal characteristics. During EMT, tumor cells undergo significant phenotypic changes, shedding epithelial features such as cell-cell adhesion and polarity. Instead, they acquire mesenchymal traits, such as enhanced motility, invasiveness, and resistance to apoptosis. The transition is driven by a network of signaling pathways and transcription factors, including Snail, ZEB, E47 and KLF8 which repress the transcription of epithelial markers like E-cadherin, and upregulate mesenchymal markers such as N-cadherin, vimentin and metalloproteases [21]. In circulation, tumor cells are referred to as circulating tumor cells (CTCs), and they must evade immune detection and extravasate to distant tissues.

Previous research has highlighted the upregulation of epithelial integrins in solid tumors, suggesting a correlation between their expression and the advancement of malignancy [22]. A single-cell RNA sequencing (scRNA-seq) analysis of CD45-negative cell fractions from breast cancer patients revealed that CTCs co-expressing ITGA6 and ITGB4 exhibited the highest frequency of tumor progression-related genes, including those associated with stemness, EMT, invasion, pro-inflammatory chemokines, and cytokines, as well as laminin subunits The Weinberg group also reported that ITGB4 can stratify mesenchymal-like human TNBC cells based on their tumorigenic abilities. Notably, ITGB4+ cancer stem cell (CSC)-enriched mesenchymal cells display an intermediate epithelial/mesenchymal phenotypic state [23]. Numerous studies continue to investigate the underlying mechanisms driving metastatic processes in ITGB4 enriched cells.

3.2. Signaling Pathways Driving Metastasis and Cell Survival

ITGB4 expression has been shown to regulate the expression of other proteins important in mediating metastasis. Gerson *et al*. found that ITGB4 potentiates the expression of secreted protein acidic and rich in cysteine (SPARC), a glycoprotein that plays an important role in matrix remodeling and invasion [24]. Another study elucidates an NFAT-dependent pathway, through which ITGB4 mediates S100 calcium binding protein A4 (S100A4) expression. S100A4 is a key protein promoting mesenchymal transition [25]. How ITGB4 enhances S100A4 gene

transcription remains to be elucidated.

Moreover, ITGB4 has been found to upregulate autotaxin expression via NFAT1, further enhancing the invasive and motile phenotype of cancer cells. Autotaxin is an autocrine motility factor that plays a significant role in chemotaxis, contributing to the metastatic spread of tumor cells [26]. Interestingly, ITGB4 has been found to transcriptionally regulate the expression of ErbB2 (also known as HER2), a receptor tyrosine kinase associated with aggressive breast cancer. This leads to the phosphorylation of the epidermal growth factor receptor (EGFR), activating the Ras signaling pathway [27]. This cascade amplifies oncogenic signaling, reinforcing the aggressive behavior of breast cancer cells.

ITGB4 expression can be affected by conditions influencing the tumor microenvironment. For example, a caveolin-1 (P132L) mutation, commonly observed in human breast cancer, promotes cell migration, invasion, and experimental metastasis, consistent with a gain-of-function mutation accompanied by increased expression of integrin signaling/adapter proteins, including ITGB4 [28].

On the other hand, UNC5C, a netrin-1 dependence receptor, appears to act as a negative regulator of ITGB4-driven metastasis. UNC5C was found to be heterogeneously expressed in breast cancer cell lines. Its knockdown in breast cancer cells enhances the expression of matrix metalloproteinases (MMPs) such as MMP3, MMP7, MMP9, and MMP10 through the activation of the PI3K/AKT, ERK, and p38 MAPK signaling pathways. This suggests that UNC5C acts as a suppressor of these signaling cascades, countering the invasive phenotype driven by ITGB4. The knockdown of UNC5C also increases the phosphorylation of FAK and SRC, key kinases involved in the netrin-1/UNC5C and netrin-1/integrin $\alpha 6/\beta 4$ signaling pathways, thereby potentiating the signaling mediated by ITGB4 [29].

Estrogen is able to enhance the aggressiveness of ERα-positive breast cancer by promoting cell proliferation, survival, EMT, and stem-like properties. ITGB4 signaling has been implicated in this process. In ERα-positive MCF-7 breast cancer cells, estrogen activates ERα transcription, inducing the expression of ΔNp63, an oncogenic truncated isoform of the p63 transcription factor. ΔNp63 functions as a transcription factor for ITGB4, leading to its increased expression. This cascade results in AKT phosphorylation, which enhances cell viability and motility, contributing to cancer progression. Conversely, in ERα-negative MDA-MB-231 cells, as well as ΔNp63 knockdown cells, estrogen does not induce the ΔNp63-ITGB4-AKT signaling pathway, highlighting the specificity of this mechanism [30].

Additionally, ITGB4 expression is maintained in cancer cells following detachment from the ECM, which is crucial for their survival during metastasis. This is in contrast to non-malignant human mammary epithelial cells (HMECs), which lose their reattachment ability and undergo non-apoptotic cell death upon detachment. The retention of ITGB4 allows cancer cells to reattach to substrata and form colonies after exposure to anchorage loss, an ability essential for establishing adhesion contact with ECM in secondary organs. This process is mediated by Rac1,

which sustains ITGB4 levels by suppressing its lysosomal degradation through an actin-related protein 2/3 (Apr2/3)- and TBC1D2-dependent pathway. Notably, the simultaneous high expression of ITGB4 and Rac1 has been associated with poor prognosis in breast cancer patients, underscoring the importance of ITGB4 in the metastatic potential of these cells [31].

Recently, Goel *et al*. found that the Hippo effectors YAP and TAZ can sustain the expression of LM332, which is the predominant ECM ligand for ITGB4 in breast carcinoma cells with epithelial differentiation. This interaction not only facilitates cellular adhesion and survival, but also plays a crucial role in the resistance to ferroptosis, a form of regulated cell death. Furthermore, ITGB4 has been shown to maintain the expression of miR-200s, which repress the transcription factor ZEB1. ZEB1 is known to influence the transcriptional activity of YAP/TAZ, and its repression enables YAP/TAZ mediated transcription of LM332, thereby enhancing the metastatic potential of cancer cells [32].

3.3. Metabolic Reprogramming

Cancer cells rely on a myriad of survival strategies to support their rapid proliferation and invasion, with metabolic reprogramming being at the forefront. A hallmark of this is the Warburg effect, where cancer cells preferentially produce ATP through aerobic glycolysis rather than oxidative phosphorylation. To compensate for the lower efficiency of glycolysis, cancer cells upregulate glucose transporters, such as GLUT1, increasing glucose uptake. This shift results in elevated glycogen synthesis and lactate production, both of which are characteristic features of cancer metabolism [33]. However, in the densely populated and nutrient-restricted TME, cancer cells face challenges in acquiring the energy and substrates necessary for synthesizing nucleotides, amino acids, and lipid precursors, which ultimately limits their proliferation.

To overcome this, cancer cells induce aerobic glycolysis in neighboring CAFs and take up the resulting products (lactate and pyruvate), using them for their own proliferation. This process, known as the reverse Warburg effect, further supports the metabolic demands of cancer cells and facilitates their invasive growth [34]. Sung *et al*. demonstrated that ITGB4-overexpressing TNBC cells provide CAFs with ITGB4 proteins via exosomal transfer, inducing BNIP3L-dependent mitophagy and lactate production in CAFs in a manner that is dependent on c-Jun or AMPK phosphorylation. The produced lactates are then exported from CAFs into the extracellular environment through the upregulated monocarboxylate transporter 4 (MCT4). Moreover, ITGB4-overexpressing CAF-conditioned medium promoted the proliferation and invasion of breast cancer cells and induced larger tumor masses in mice with CAFs compared to those with ITGB4 knockdown tumors. This work reveals how TNBC-derived ITGB4 protein triggers glycolysis in CAFs and suggests that targeting ITGB4-induced mitophagy could be a promising cancer therapy strategy [35].

The signaling pathways involving ITGB4 are critical in modulating cancer cell

behavior and survival, which involves its interactions with other signaling molecules. Understanding these pathways offers potential insight into cancer interventions. Zhao *et al*. elucidated the role of ITGB4 in reprogramming lipid metabolism within liver cancer cells via the EGFR/ITGB4/SREBP1c pathway [36]. Their research revealed that this pathway is intricately linked to the expression of programmed death-ligand 1 (PD-L1), a key co-inhibitory checkpoint molecule that suppresses T-cell activity against tumor cells. The study found that PD-L1 forms a complex with EGFR and ITGB4, which in turn activates the PI3K/mTOR/SREBP1c signaling cascade. This activation leads to the reprogramming of lipid metabolism within the tumor cells, a process that is crucial for sustaining the energy demands of rapidly proliferating cancer cells and contributing to tumor growth and survival. In this context, Wang *et al*. demonstrated that Ziyuglycoside II, the main compound extracted from *Sanguisorba Officinalis* L., suppresses the aggressive phenotype of TNBC cells by targeting the Src/EGFR-dependent ITGB4/FAK signaling axis. This suggests similar mechanisms may be present in breast cancer, highlighting the importance of metabolic pathways in cancer progression [37].

3.4. Angiogenesis

Tumor-induced angiogenesis is a critical parameter of tumorigenesis and is important for tumor growth and metastasis. Recent studies provide evidence for the role of a pvrl-4 encoded gene, nectin-4, in tumorigenesis and angiogenesis. Nectin-4 is exclusively expressed in cancer cells. In a recent study by expounding this pathway, Siddharth *et al*. found that in the case of hypoxia, metastatic breast cancer stem cells (mBCSCs) secrete various diffusible factors, including the ecto-domain of NECTIN-4. This ecto-domain physically interacts with ITGB4 on endothelial cells, triggering an angiogenesis cascade via the Src, PI3K, AKT, and iNOS pathways. Disrupting this interaction effectively abrogates mBSCS-induced angiogenesis, highlighting a potential therapeutic strategy for targeting the angiogenic mechanisms driven by breast cancer [38]. However, pharmacological disruption of protein-protein interactions has generally proven challenging.

3.5. Immune System Modulation

The interaction between ITGB4 and the immune system in breast cancer is a new area of investigation. ITGB4 expression in tumor cells can influence the immune microenvironment by modulating the activity and recruitment of immune cells. The integrin has been shown to affect the infiltration of tumor-associated macrophages (TAMs), which play a dual role in cancer progression.

TAMs are known to exhibit plasticity, meaning they can either support antitumor immunity (M1-like phenotype) or promote tumor growth and metastasis (M2-like phenotype) depending on their polarization state [39]. The presence of ITGB4 in tumor cells has been linked to the modulation of TAM infiltration, potentially skewing these cells towards a tumor-promoting phenotype, which supports metastasis, angiogenesis, and immunosuppression within the tumor

microenvironment [22].

A recent study by Miyako *et al*. investigated the relationship between periostin (POSTN), a protein highly expressed in CAF-like cells, and its receptor ITGB4 in esophageal squamous cell carcinoma (ESCC). The study demonstrated that knockdown of ITGB4 resulted in the inhibition of Akt and Erk phosphorylation, critical pathways that drive the survival and migration of POSTN-enriched cells. Moreover, ITGB4 knockdown reduced the migration of mesenchymal stem cells and macrophages, effectively diminishing their TAM-like properties [40]. However, targeting POSTN for cancer therapy may prove problematic as many normal human tissues express high levels of this protein, such as the cerebral cortex, lung, fallopian tube, and breast.

Additionally, ITGB4-mediated signaling may impact the expression of immune checkpoint molecules, such as PD-L1, thereby affecting the immune system's ability to recognize and attack tumor cells. A study by Wang *et al*. found that in breast and prostate cancer cells, PD-L1 concentrates at the rear of migrating carcinoma cells, where it facilitates retraction by interacting with ITGB4, resulting in the formation of PD-L1-containing retraction fibers and migrasomes. This mechanism involves PD-L1's ability to maintain cell polarity and lower membrane tension at the cell rear, compared to the leading edge, promoting the localized interaction of PD-L1 with ITGB4 and RhoA-mediated contractility [41].

PD-L1 can also promote the growth and metastasis of cervical cancer by activating the ITGB4/SNAI1/SIRT3 signaling pathway. Mechanistically, PD-L1 binds directly to ITGB4, activating the AKT/GSK3β signaling pathway and consequently inducing the expression of the transcriptional repressor SNAI1. SNAIL, in turn, influences the expression of genes involved in EMT, further driving tumor invasiveness. Additionally, SNAIL regulates glucose metabolism by inhibiting the activity of the SIRT3 promoter, linking metabolic reprogramming with cancer progression through ITGB4 signaling [42]. Similar findings were made in liver cancer where tumor cell-expressed PD-L1 reprograms lipid metabolism via EGFR/ITGB4/SREBP1c signaling [36]. Understanding these interactions is essential for developing immunotherapeutic strategies that can effectively target ITGB4-positive breast cancer cells.

4. Drug Resistance

TNBC, the most lethal type of breast cancer, grows rapidly, often evading detection during routine mammograms and metastasizing at an early stage. Although initially responsive to chemotherapy, TNBC frequently recurs and metastasizes due to the development of chemoresistance [43]. This resistance emerges from several mechanisms, including enhanced DNA damage repair, reduced intracellular drug accumulation, and elevated anti-apoptotic activities. As a result, current treatments for TNBC patients primarily involve a combination of cytotoxic chemotherapies, surgery, and radiation, with newer approaches incorporating poly (ADP-ribose) polymerase (PARP) inhibitors and immunotherapy [44].

As described previously, ITGB4 overexpression enhances cell survival pathways and facilitates the invasive behavior of cancer cells, promoting drug resistance. Understanding and therapeutically targeting ITGB4-driven processes can outmaneuver the adaptive capabilities of TNBC cells, potentially improving patient outcomes. Weaver *et al*. discovered that mammary epithelial cells could evade apoptosis by forming polarized, three-dimensional structures, a process driven by ITGB4. This structural organization, along with ITGB4-dependent activation of NF-kB, helps protect the cells from apoptosis-inducing agents [45].

Recent studies demonstrate that TNFAIP2, which is abnormally expressed in TNBC, activates RAC1 to promote TNBC cell proliferation and migration [46]. Furthermore, Fang *et al*. found that TNFAIP2 interacts with IQGAP1 and ITGB4. ITGB4 activates RAC1, a member of the Ras family of GTPases, through TNFAIP2 and IQGAP1 and confers DNA damage-related drug resistance in TNBC to epirubicin, cisplatin, talazoparib, and olaparib [47].

However, it is important to note another study that found ITGB4 could promote sensitivity to cisplatin and carboplatin, rather than contribute to resistance. The study revealed that ITGB4 signaling activates key components of the DNA damage response pathway, including mutant p53 (common in TNBC), ATM and 53BP1, a signaling cascade that enhances the effectiveness of cisplatin. Additionally, ITGB4 was shown to suppress homologous recombination (HR) and boost non-homologous end joining (NHEJ) repair in response to cisplatin-induced DNA double-strand breaks. When HR is inhibited, cells rely more on the less accurate NHEJ repair pathway, leading to an accumulation of DNA damage. This discovery highlights the complex and context-dependent role of integrin signaling in drug resistance and sensitivity [48].

Overall, these studies suggest that the ITGB4 signaling axis may provide potential therapeutic targets to overcome chemoresistance in TNBC.

5. Therapeutic Targeting of ITGB4

Several studies have explored therapeutic strategies targeting ITGB4. Ruan *et al*. developed two immunologic approaches to target ITGB4: vaccination with ITGB4 protein-pulsed dendritic cells (ITGB4-DC) and the adoptive transfer of anti-CD3/anti-ITGB4 bispecific antibody (ITGB4 BiAb)-armed tumor-draining lymph node T cells. These strategies were tested in two immunocompetent mouse models—4T1 mammary tumors and SCC7 head and neck squamous carcinoma—to evaluate their effectiveness in targeting both CSCs and bulk tumor populations. Both methods significantly inhibited local tumor growth and metastasis in these models, with efficacy further enhanced by the addition of anti-PD-L1. Importantly, both ITGB4-targeted immunotherapies induced T-cell cytotoxicity against both CSCs and non-CSCs expressing ITGB4, without any apparent toxicity [49]. However, a closer examination of the data presented in this study reveals some interesting observations. a) Depleting ITGB4 expression (KO) in 4T1 cells had similar effects on tumor growth as transferring ITGB4-BiAb-armed

tumor-draining lymph node (TDLN) T cells; b) There was no additive effects of combining the approaches of ITGB4 KO and BiAb-armed TDLN T cell treatment. These data suggest that targeting ITGB4 in tumor cells alone may be sufficient to trigger an anti-tumor response without the help of the BiAb-armed T cells.

In a more recent study, Goel *et al*. showed that ITGB4 repression of ZEB1 promotes ferroptosis resistance, indicating that modulating ITGB4 activity could enhance the effectiveness of ferroptosis-inducing therapies [32]. These findings support the potential of ITGB4-targeted immunotherapy as a promising strategy across various tumor types and suggest that combining ITGB4 targeting with ferroptosis-inducing treatments could offer a synergistic approach to improving cancer therapy outcomes.

ARRDC3, a member of the mammalian α-arrestins family, has been identified as a tumor suppressor in breast cancer. Zheng *et al*. investigated the role of ARRDC in prostate cancer, and found its expression was associated with higher Gleason scores, metastasis, and biochemical recurrence. Notably, ARRDC3 expression was negatively correlated with ITGB4 levels in clinical prostate cancer samples. The team demonstrated that inhibiting the ARRDC3-ITGB4 pathway could suppress prostate cancer progression, suggesting a similar approach might be applicable to breast cancer [50].

As discussed previously, ITGB4 has been found to regulate HER2 expression, with HER2 being an oncogenic receptor overexpressed in 25 - 30% of breast cancer patients [51]. Recent studies have investigated the potential of Cucurbitacin B (CuB), a triterpenoid steroidal compound, as a therapeutic agent for breast cancer, particularly in HER2-positive cases. CuB was found to effectively inhibit breast cancer cell growth, significantly suppressing HER2 and integrin signaling. CuB treatment also upregulated integrins ITGB1 and ITGB3, known to induce integrin-mediated cell death. In vivo experiments using orthotopic models with MDA-MB-231 and 4T-1 breast cancer cells demonstrated that CuB reduced tumor growth by 55% and 40%, respectively, suggesting at CuB may offer an innovative therapeutic approach by targeting HER2 and integrin signaling in breast cancer [52].

6. Conclusion

ITGB4 plays a multifaceted role in breast cancer progression. Its overexpression is strongly correlated with aggressive and invasive cancer phenotypes, particularly in TNBC, where it can serve as a potential prognostic marker. ITGB4, expressed principally by endothelial and epithelial cells, promotes tumor invasion, metastasis, and survival by facilitating EMT, reprogramming cellular metabolism, and interacting with the immune microenvironment. Its involvement in crucial signaling pathways, such as those involving EGFR and NFAT, as well as its role in modulating the tumor microenvironment, highlights its significance in breast cancer progression and resistance to therapy. To date, no ITGB4-specific pharmacological inhibitors have been developed. Nevertheless, targeting ITGB4-mediated pathways

by creative means could offer novel therapeutic strategies to combat the aggressive nature of certain breast cancers, although serious considerations must be given to ways to limit the potential toxicity of such innovative therapeutic agents, given the fact that many normal tissues also express ITGB4 to varying degrees.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Nicolis, O., De Los Angeles, D. and Taramasco, C. (2024) A Contemporary Review of Breast Cancer Risk Factors and the Role of Artificial Intelligence. *Frontiers in Oncology*, **14**, Article 1356014. https://doi.org/10.3389/fonc.2024.1356014

[2] Takada, Y., Ye, X. and Simon, S. (2007) The Integrins. *Genome Biology*, **8**, Article No. 215. https://doi.org/10.1186/gb-2007-8-5-215

[3] Li, J., Sun, H., Feltri, M.L. and Mercurio, A.M. (2015) Integrin $\beta 4$ Regulation of PTHrP Underlies Its Contribution to Mammary Gland Development. *Developmental Biology*, **407**, 313-320. https://doi.org/10.1016/j.ydbio.2015.09.015

[4] Walker, M.R., Amante, J.J., Li, J., Liu, H., Zhu, L.J., Feltri, M.L., *et al.* (2020) Alveolar Progenitor Cells in the Mammary Gland Are Dependent on the $\beta 4$ Integrin. *Developmental Biology*, **457**, 13-19. https://doi.org/10.1016/j.ydbio.2019.10.001

[5] Yang, H., Xu, Z., Peng, Y., Wang, J. and Xiang, Y. (2021) Integrin $\beta 4$ as a Potential Diagnostic and Therapeutic Tumor Marker. *Biomolecules*, **11**, Article 1197. https://doi.org/10.3390/biom11081197

[6] Lipscomb, E.A. and Mercurio, A.M. (2005) Mobilization and Activation of a Signaling Competent $\alpha 6\beta 4$ Integrin Underlies Its Contribution to Carcinoma Progression. *Cancer and Metastasis Reviews*, **24**, 413-423. https://doi.org/10.1007/s10555-005-5133-4

[7] Soung, Y.H., Ford, S., Yan, C. and Chung, J. (2018) The Role of Arrestin Domain-Containing 3 in Regulating Endocytic Recycling and Extracellular Vesicle Sorting of Integrin $\beta 4$ in Breast Cancer. *Cancers*, **10**, Article 507. https://doi.org/10.3390/cancers10120507

[8] Bierie, B., Pierce, S.E., Kroeger, C., Stover, D.G., Pattabiraman, D.R., Thiru, P., *et al.* (2017) Integrin-$\beta 4$ Identifies Cancer Stem Cell-Enriched Populations of Partially Mesenchymal Carcinoma Cells. *Proceedings of the National Academy of Sciences of the United States of America*, **114**, E2337-E2346. https://doi.org/10.1073/pnas.1618298114

[9] Hoshino, A., Costa-Silva, B., Shen, T., Rodrigues, G., Hashimoto, A., Tesic Mark, M., *et al.* (2015) Tumour Exosome Integrins Determine Organotropic Metastasis. *Nature*, **527**, 329-335. https://doi.org/10.1038/nature15756

[10] Ma, B., Zhang, L., Zou, Y., He, R., Wu, Q., Han, C., *et al.* (2019) Reciprocal Regulation

of Integrin β4 and KLF4 Promotes Gliomagenesis through Maintaining Cancer Stem Cell Traits. *Journal of Experimental & Clinical Cancer Research*, **38**, Article No. 23. https://doi.org/10.1186/s13046-019-1034-1

[11] Ni, H., Dydensborg, A.B., Herring, F.E., Basora, N., Gagné, D., Vachon, P.H., *et al*. (2005) Upregulation of a Functional Form of the B4 Integrin Subunit in Colorectal Cancers Correlates with C-Myc Expression. *Oncogene*, **24**, 6820-6829. https://doi.org/10.1038/sj.onc.1208848

[12] Aslan, B., Monroig, P., Hsu, M., Pena, G.A., Rodriguez-Aguayo, C., Gonzalez-Villasana, V., *et al*. (2015) The ZNF304-Integrin Axis Protects against Anoikis in Cancer. *Nature Communications*, **6**, Article No. 7351. https://doi.org/10.1038/ncomms8351

[13] Bon, G., Di Carlo, S.E., Folgiero, V., Avetrani, P., Lazzari, C., D'Orazi, G., *et al*. (2009) Negative Regulation of β4 Integrin Transcription by Homeodomain-Interacting Protein Kinase 2 and P53 Impairs Tumor Progression. *Cancer Research*, **69**, 5978-5986. https://doi.org/10.1158/0008-5472.can-09-0244

[14] Huang, W., Fan, L., Tang, Y., Chi, Y. and Li, J. (2021) A Pan-Cancer Analysis of the Oncogenic Role of Integrin β4 (ITGB4) in Human Tumors. *International Journal of General Medicine*, **14**, 9629-9645. https://doi.org/10.2147/ijgm.s341076

[15] Li, J., Jiang, Y., Chen, C., Tan, W., Li, P., Chen, G., *et al*. (2020) Integrin β4 Is an Effective and Efficient Marker in Synchronously Highlighting Lymphatic and Blood Vascular Invasion, and Perineural Aggression in Malignancy. *American Journal of Surgical Pathology*, **44**, 681-690. https://doi.org/10.1097/pas.0000000000001451

[16] Brendle, A., Lei, H., Brandt, A., Johansson, R., Enquist, K., Henriksson, R., *et al*. (2008) Polymorphisms in Predicted microRNA-Binding Sites in Integrin Genes and Breast Cancer: ITGB4 as Prognostic Marker. *Carcinogenesis*, **29**, 1394-1399. https://doi.org/10.1093/carcin/bgn126

[17] Walko, G., Castañón, M.J. and Wiche, G. (2015) Molecular Architecture and Function of the Hemidesmosome. *Cell and Tissue Research*, **360**, 529-544. https://doi.org/10.1007/s00441-015-2216-6

[18] Rabinovitz, I., Toker, A. and Mercurio, A.M. (1999) Protein Kinase C–dependent Mobilization of the α6β4 Integrin from Hemidesmosomes and Its Association with Actin-Rich Cell Protrusions Drive the Chemotactic Migration of Carcinoma Cells. *The Journal of Cell Biology*, **146**, 1147-1160. https://doi.org/10.1083/jcb.146.5.1147

[19] Stewart, R.L. and O'Connor, K.L. (2015) Clinical Significance of the Integrin α6β4 in Human Malignancies. *Laboratory Investigation*, **95**, 976-986. https://doi.org/10.1038/labinvest.2015.82

[20] Balkwill, F.R., Capasso, M. and Hagemann, T. (2012) The Tumor Microenvironment at a Glance. *Journal of Cell Science*, **125**, 5591-5596. https://doi.org/10.1242/jcs.116392

[21] Thiery, J.P., Acloque, H., Huang, R.Y.J. and Nieto, M.A. (2009) Epithelial-Mesenchymal Transitions in Development and Disease. *Cell*, **139**, 871-890. https://doi.org/10.1016/j.cell.2009.11.007

[22] Evans, R., Flores-Borja, F., Nassiri, S., Miranda, E., Lawler, K., Grigoriadis, A., *et al*. (2019) Integrin-Mediated Macrophage Adhesion Promotes Lymphovascular Dissemination in Breast Cancer. *Cell Reports*, **27**, 1967-1978.e4. https://doi.org/10.1016/j.celrep.2019.04.076

[23] Grigoryeva, E., Tashireva, L., Alifanov, V., Savelieva, O., Zavyalova, M., Menyailo, M., *et al*. (2024) Integrin-Associated Transcriptional Characteristics of Circulating

Tumor Cells in Breast Cancer Patients. *PeerJ*, **12**, e16678. https://doi.org/10.7717/peerj.16678

[24] Gerson, K.D., Shearstone, J.R., Maddula, V.S.R.K., Seligmann, B.E. and Mercurio, A.M. (2012) Integrin β4 Regulates SPARC Protein to Promote Invasion. *Journal of Biological Chemistry*, **287**, 9835-9844. https://doi.org/10.1074/jbc.m111.317727

[25] Kim, T.H., Kim, H.I., Soung, Y.H., Shaw, L.A. and Chung, J. (2009) Integrin (α6β4) Signals through SRC to Increase Expression of S100A4, a Metastasis-Promoting Factor: Implications for Cancer Cell Invasion. *Molecular Cancer Research*, **7**, 1605-1612. https://doi.org/10.1158/1541-7786.mcr-09-0102

[26] Chen, M. and O'Connor, K.L. (2005) Integrin α6β4 Promotes Expression of Autotaxin/ENPP2 Autocrine Motility Factor in Breast Carcinoma Cells. *Oncogene*, **24**, 5125-5130. https://doi.org/10.1038/sj.onc.1208729

[27] Yoon, S., Shin, S. and Lipscomb, E.A. (2006) A Novel Mechanism for Integrin-Mediated Ras Activation in Breast Carcinoma Cells: The α6β4 Integrin Regulates ErbB2 Translation and Transactivates Epidermal Growth Factor Receptor/ErbB2 Signaling. *Cancer Research*, **66**, 2732-2739. https://doi.org/10.1158/0008-5472.can-05-2941

[28] Bonuccelli, G., Casimiro, M.C., Sotgia, F., Wang, C., Liu, M., Katiyar, S., *et al*. (2009) Caveolin-1 (P132L), a Common Breast Cancer Mutation, Confers Mammary Cell Invasiveness and Defines a Novel Stem Cell/Metastasis-Associated Gene Signature. *The American Journal of Pathology*, **174**, 1650-1662. https://doi.org/10.2353/ajpath.2009.080648

[29] Yuan, M.J., *et al*. (2024) UNC5C Knockdown Enhances the Growth and Metastasis of Breast Cancer Cells by Potentiating the Integrin α6/β4 Signaling Pathway. *International Journal of Oncology*, **56**, 139-150. https://www.spandidos-publications.com/10.3892/ijo.2019.4931

[30] Ho, J., Chang, F., Huang, F.S., Liu, J., Liu, Y., Chen, S., *et al*. (2016) Estrogen Enhances the Cell Viability and Motility of Breast Cancer Cells through the ERα-δNp63-Integrin β4 Signaling Pathway. *PLOS ONE*, **11**, e0148301. https://doi.org/10.1371/journal.pone.0148301

[31] Mori, K., Higurashi, M., Ishikawa, F. and Shibanuma, M. (2021) Rac1-Mediated Sustained β4 Integrin Level Develops Reattachment Ability of Breast Cancer Cells after Anchorage Loss. *Cancer Science*, **112**, 3205-3217. https://doi.org/10.1111/cas.14985

[32] Goel, H.L., Karner, E.R., Kumar, A., Mukhopadhyay, D., Goel, S. and Mercurio, A.M. (2024) YAP/TAZ-Mediated Regulation of Laminin 332 Is Enabled by β4 Integrin Repression of ZEB1 to Promote Ferroptosis Resistance. *Journal of Biological Chemistry*, **300**, Article ID: 107202. https://doi.org/10.1016/j.jbc.2024.107202

[33] DeBerardinis, R.J., Lum, J.J., Hatzivassiliou, G. and Thompson, C.B. (2008) The Biology of Cancer: Metabolic Reprogramming Fuels Cell Growth and Proliferation. *Cell Metabolism*, **7**, 11-20. https://doi.org/10.1016/j.cmet.2007.10.002

[34] Pavlides, S., Whitaker-Menezes, D., Castello-Cros, R., Flomenberg, N., Witkiewicz, A.K., Frank, P.G., *et al*. (2009) The Reverse Warburg Effect: Aerobic Glycolysis in Cancer Associated Fibroblasts and the Tumor Stroma. *Cell Cycle*, **8**, 3984-4001. https://doi.org/10.4161/cc.8.23.10238

[35] Sung, J.S., Kang, C.W., Kang, S., Jang, Y., Chae, Y.C., Kim, B.G., *et al*. (2019) Itgb4-mediated Metabolic Reprogramming of Cancer-Associated Fibroblasts. *Oncogene*, **39**, 664-676. https://doi.org/10.1038/s41388-019-1014-0

[36] Zhao, M., Yuan, H., Yang, G., Wang, Y., Bu, Y., Zhang, H., *et al*. (2024) Tumour Cell-

Expressed PD-L1 Reprograms Lipid Metabolism via EGFR/ITGB4/SREBP1c Signalling in Liver Cancer. *JHEP Reports*, **6**, Article ID: 101009. https://doi.org/10.1016/j.jhepr.2024.101009

[37] Wang, K., Zou, P., Zhu, X. and Zhang, T. (2019) Ziyuglycoside II Suppresses the Aggressive Phenotype of Triple Negative Breast Cancer Cells through Regulating SRC/EGFR-Dependent ITGB4/FAK Signaling. *Toxicology in Vitro*, **61**, Article ID: 104653. https://doi.org/10.1016/j.tiv.2019.104653

[38] Siddharth, S., Nayak, A., Das, S., Nayak, D., Panda, J., Wyatt, M.D., *et al*. (2018) The Soluble Nectin-4 Ecto-Domain Promotes Breast Cancer Induced Angiogenesis via Endothelial Integrin-β4. *The International Journal of Biochemistry & Cell Biology*, **102**, 151-160. https://doi.org/10.1016/j.biocel.2018.07.011

[39] Boutilier, A.J. and Elsawa, S.F. (2021) Macrophage Polarization States in the Tumor Microenvironment. *International Journal of Molecular Sciences*, **22**, Article 6995. https://doi.org/10.3390/ijms22136995

[40] Miyako, S., Koma, Y., Nakanishi, T., Tsukamoto, S., Yamanaka, K., Ishihara, N., *et al*. (2024) Periostin in Cancer-Associated Fibroblasts Promotes Esophageal Squamous Cell Carcinoma Progression by Enhancing Cancer and Stromal Cell Migration. *The American Journal of Pathology*, **194**, 828-848. https://doi.org/10.1016/j.ajpath.2023.12.010

[41] Wang, M., Xiong, C. and Mercurio, A.M. (2022) PD-LI Promotes Rear Retraction during Persistent Cell Migration by Altering Integrin β4 Dynamics. *Journal of Cell Biology*, **221**, e202108083. https://doi.org/10.1083/jcb.202108083

[42] Wang, S., Li, J., Xie, J., Liu, F., Duan, Y., Wu, Y., *et al*. (2018) Programmed Death Ligand 1 Promotes Lymph Node Metastasis and Glucose Metabolism in Cervical Cancer by Activating Integrin β4/SNAI1/SIRT3 Signaling Pathway. *Oncogene*, **37**, 4164-4180. https://doi.org/10.1038/s41388-018-0252-x

[43] Jamdade, V.S., Sethi, N., Mundhe, N.A., Kumar, P., Lahkar, M. and Sinha, N. (2015) Therapeutic Targets of Triple-Negative Breast Cancer: A Review. *British Journal of Pharmacology*, **172**, 4228-4237. https://doi.org/10.1111/bph.13211

[44] Hill, D.P., Harper, A., Malcolm, J., McAndrews, M.S., Mockus, S.M., Patterson, S.E., *et al*. (2019) Cisplatin-Resistant Triple-Negative Breast Cancer Subtypes: Multiple Mechanisms of Resistance. *BMC Cancer*, **19**, Article No. 1039. https://doi.org/10.1186/s12885-019-6278-9

[45] Weaver, V.M., Lelièvre, S., Lakins, J.N., Chrenek, M.A., Jones, J.C.R., Giancotti, F., *et al*. (2002) β4 Integrin-Dependent Formation of Polarized Three-Dimensional Architecture Confers Resistance to Apoptosis in Normal and Malignant Mammary Epithelium. *Cancer Cell*, **2**, 205-216. https://doi.org/10.1016/s1535-6108(02)00125-3

[46] Jia, L., Zhou, Z., Liang, H., Wu, J., Shi, P., Li, F., *et al*. (2015) KLF5 Promotes Breast Cancer Proliferation, Migration and Invasion in Part by Upregulating the Transcription of TNFAIP2. *Oncogene*, **35**, 2040-2051. https://doi.org/10.1038/onc.2015.263

[47] Fang, H., Ren, W., Cui, Q., Liang, H., Yang, C., Liu, W., *et al*. (2023) Integrin β4 Promotes DNA Damage-Related Drug Resistance in Triple-Negative Breast Cancer via TNFAIP2/IQGAP1/RAC1. *eLife*, **12**, RP88483. https://doi.org/10.7554/elife.88483

[48] Chen, M., Marrs, B., Qi, L., Knifley, T., Weiss, H.L., D'Orazio, J.A., *et al*. (2022) Integrin $\alpha6\beta4$ Signals through DNA Damage Response Pathway to Sensitize Breast Cancer Cells to Cisplatin. *Frontiers in Oncology*, **12**, Article 1043538. https://doi.org/10.3389/fonc.2022.1043538

[49] Ruan, S., Lin, M., Zhu, Y., Lum, L., Thakur, A., Jin, R., *et al*. (2020) Integrin β4-Targeted Cancer Immunotherapies Inhibit Tumor Growth and Decrease Metastasis. *Cancer Research*, **80**, 771-783. https://doi.org/10.1158/0008-5472.can-19-1145

[50] Zheng, Y., Lin, Z.Y., Xie, J.J., Jiang, F.N., Chen, C.J., Li, J.X., *et al*. (2017) ARRDC3 Inhibits the Progression of Human Prostate Cancer through ARRDC3-ITGβ4 Pathway. *Current Molecular Medicine*, **17**, 221-229. https://doi.org/10.2174/1566524017666170807144711

[51] Vivek, R., Rejeeth, C. and Thangam, R. (2017) Targeted Nanotherapeutics Based on Cancer Biomarkers. In: Grumezescu, A.M., Ed., *Multifunctional Systems for Combined Delivery*, *Biosensing and Diagnostics*, Elsevier, 229-244. https://doi.org/10.1016/b978-0-323-52725-5.00012-5

[52] Gupta, P. and Srivastava, S.K. (2014) Inhibition of HER2-Integrin Signaling by Cucurbitacin B Leads to *in Vitro* and *in Vivo* Breast Tumor Growth Suppression. *Oncotarget*, **5**, 1812-1828. https://doi.org/10.18632/oncotarget.1743