

Review

Integrin $\alpha 5\beta 1$, the Fibronectin Receptor, as a Pertinent Therapeutic Target in Solid Tumors

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Abstract: Integrins are transmembrane heterodimeric proteins sensing the cell microenvironment and modulating numerous signalling pathways. Changes in integrin expression between normal and tumoral cells support involvement of specific integrins in tumor progression and aggressiveness. This review highlights the current knowledge about $\alpha 5\beta 1$ integrin, also called the fibronectin receptor, in solid tumors. We summarize data showing that $\alpha 5\beta 1$ integrin is a pertinent therapeutic target expressed by tumoral neovessels and tumoral cells. Although mainly evaluated in preclinical models, $\alpha 5\beta 1$ integrin merits interest in particular in colon, breast, ovarian, lung and brain tumors where its overexpression is associated with a poor prognosis for patients. Specific $\alpha 5\beta 1$ integrin antagonists will be listed that may represent new potential therapeutic agents to fight defined subpopulations of particularly aggressive tumors.

Keywords: $\alpha 5\beta 1$; integrin; fibronectin receptor; solid tumors; angiogenesis; antagonists

1. Introduction

In recent years, integrins have attracted increasing interest for their potential to act as tumor therapeutic targets [1,2]. First recognized as cell adhesion molecules and receptors for the extracellular matrix (ECM), it is now widely acknowledged that integrins act as true receptors regulating intracellular signaling and cellular responses including migration, proliferation and differentiation [3].

Integrins are $\alpha\beta$ protein heterodimers whose non covalent association defines the specificity of adhesion to particular components of the ECM or other proteins (immunoglobulin superfamily molecules, plasma proteins, VCAM1, *etc.*). In mammals, 18 α subunits and eight β subunits form a large family of about 24 $\alpha\beta$ integrins, which bind to tissue and organ specific ligands. Regulating the crosstalk between cells and their surrounding microenvironment, integrins are particularly relevant in different key aspects of tumor progression. Depending on the tumor types, the expression of specific integrins differs between tumoral tissues and their corresponding healthy tissues. Integrins are overexpressed in cancer cells impacting proliferation, survival, resistance to therapies and tumor recurrence. In addition, an important role in tumor angiogenesis has been highlighted for several integrins including $\alpha5\beta1$ and $\alpha v\beta3/\beta5$. Their overexpression on neo-vessels during the tumor angiogenic switch led to the suggestion of new anti-angiogenic therapies [4]. Cilengitide, a specific antagonist for $\alpha v\beta3/\beta5$ integrins is currently in clinical trials for the treatment of different tumors as for example the highly vascularized brain tumor glioblastoma [5]. Different recent reviews have already summarized the current knowledge about integrin structure, activation and signaling as well as integrin implication as therapeutic targets in cancer and/or angiogenesis [2–4,6–9]. The goal of this review is to focus on $\alpha5\beta1$ integrin, also called the fibronectin receptor, as an emerging therapeutic target in different solid tumors. The role of $\alpha5\beta1$ integrin in cancer has been somewhat controversial with data suggesting tumor suppressive effects while others are in favor of a protumoral behavior. In the last decade, the relationship between $\alpha5\beta1$ integrin expression on tumors and patient survival has become increasingly recognized in several cancers. In this article we provide an overview of the implication of $\alpha5\beta1$ integrin in tumor angiogenesis, and solid tumor aggressiveness and we list the currently available $\alpha5\beta1$ integrin antagonists.

2. Generalities on $\alpha5\beta1$ Integrin

Integrins are composed of an extracellular domain, a transmembrane domain and a short cytoplasmic tail. The α subunit extracellular domain has a 7-bladed β propeller connected to a thigh and two calf domains. In addition nine of the α subunits have an I domain that contains the metal ion-dependent adherent site (MIDAS), crucial for ligand binding [10]. The α subunit is responsible for the high specificity of $\alpha5\beta1$ integrin for fibronectin. The combination of a primary interaction with the RGD site with a secondary interaction with the synergy site of fibronectin ensures both high affinity and specificity of $\alpha5\beta1$ integrin for fibronectin [11,12]. A recent crystallography structure of $\alpha5\beta1$ integrin in complex with a RGD-peptide has indicated that interaction of this integrin with the synergy site comes from the residue Asp 154 in the $\alpha5$ subunit and also that Ca^{2+} is an important cation for fibronectin binding [13]. The β subunit extracellular part is composed of a hybrid domain, a beta1 domain (with a MIDAS structure), a plexin/semaphorin/integrin domain and four EGF like domains [14].

Crystal structures have revealed that $\beta3$ integrins occur in three possible states: low, intermediate and high affinity for its ligand [15,16]. Activation of integrin either by binding to its ligand (outside-in signaling) or by binding of an activator protein like talin to its cytoplasmic tail (inside-out signaling) result in a change in conformation from a bent (low affinity) to an extended (high affinity). At the proximal site of the transmembrane domain GFFKR, residues, extremely conserved in the α subunit, and hydrophobic membrane-proximal residues in the β subunit, are involved in releasing the integrin

from an inactive to an active conformation, with unbound cytoplasmic tails available for signal transduction [7,17]. Integrins do not have enzymatic activity therefore signal transduction is performed by proteins recruited to the cytoplasmic tail such as FAK, src, and talin [1,7]. Length and intensity of the signal is regulated in part by specific negative regulators proteins such as ICAP1 or sharpin [18]. Integrin $\alpha 5\beta 1$ has specific regulator such as nischarin [19] and GIPC1 [20]. In addition to the recruitment of signaling inhibitors, integrin trafficking can regulate signaling [21]. After activation, $\alpha 5\beta 1$ integrin can either be ubiquitinated and degraded in lysosome or get recycled rapidly to the plasma membrane via the early endosome pathway [21]. Ubiquitination and degradation in lysosome of $\alpha 5\beta 1$ integrin is important for proper fibroblast migration on fibronectin [22]. Integrins can also be internalized via clathrin dependent or independent pathway [21]. Caveolar internalization of $\alpha 5\beta 1$ integrin and fibronectin promotes matrix turnover [23]. Interestingly, it seems that caveolin-1 is capable of internalizing $\alpha 5\beta 1$ integrin without fibronectin, thereby regulating the amount of the fibronectin receptor available on the cell surface. This type of regulation was also shown on endothelial cell with internalization of active or inactive $\alpha 5\beta 1$ integrin by two different pathways [20]. Rapid recycling of $\beta 1$ integrin to the plasma membrane through early endosome is regulated by Rab5-related GTPase Rab21 binding to a region close to the GFFKR motif [24]. Other small GTPase protein regulates $\alpha 5\beta 1$ integrin like Rab25 that promotes invasion in cancer cells [25–27]. Recent data also point to a role of $\alpha 5\beta 1$ integrin in mechanosensing [28,29]. In response to mechanical forces, $\alpha 5\beta 1$ integrin switches between relaxed and tensioned states which allow strong adhesion and downstream signals [30,31]. The synergy site in fibronectin is required to form the tensioned bonds although the relaxed bonds only involve the RGD site [31]. Mechanical stimulation of $\alpha 5\beta 1$ integrin enhances cancer cell invasion [32,33]. In summary, integrin bidirectional signaling regulates several processes such as migration, invasion, proliferation and survival specific to each cell types and is a major regulator of cancer progression which will be discussed next [1,6,8].

3. Integrin $\alpha 5\beta 1$ and Angiogenesis

Angiogenesis is a key physiological and pathological process that is regulated in part by integrins. Integrins are expressed by endothelial cells, smooth muscle cells and cellular components of the blood such as platelets, monocytes, lymphocytes *etc.* for example [4]. Among the large integrin family, $\alpha 4$, $\alpha 5$, αv or $\beta 1$ integrin subunits were shown to be required for vasculo- and angiogenesis during development [34–36]. Yet, each of these integrins have specific function: $\alpha 4$ knock out mice display an abnormal retention of hematopoietic stem cells in the bone marrow niche and cardiac defect, αv knock out mice show predominantly hemorrhage in the brain and intestine resulting in death from mid-gestation to perinatal, and $\beta 1$ knock out specifically in endothelial cells resulting in the most severe phenotype with vascular remodeling defects caused by adhesion and migration alteration and reduced survival of endothelial cells. Homozygous deletion of $\alpha 5$ integrin subunit results in embryonic death at around E 10.5 due to defects in posterior trunk development (defects in neural tube and somites), and angiogenesis [37,38]. The $\alpha 5$ null embryos and the extraembryonic vessels display defects resulting in leakage of blood cells. Angiogenesis abnormalities are also observed in mice deficient in fibronectin, the major ligand for $\alpha 5\beta 1$ integrin, although lethality occurs earlier at E9.5 [39]. To further understand the mechanism regulated by $\alpha 5$ integrin subunit in angiogenesis, conditional

knock out were designed where $\alpha 5^{\text{flox/flox}}$ mice were crossed with Tie2-Cre mice. The resulting mice do not express $\alpha 5$ integrin subunit on endothelial and hematopoietic cells. Surprisingly the embryos developed normally and do not harbor angiogenesis defects [40]. Analysis of adhesion of $\alpha 5$ null endothelial cells shows that αv is present to focal adhesion points and therefore compensates for the lack of $\alpha 5$. Double knock out of $\alpha 5$ and αv in endothelial cells results in abnormal vessel remodeling and heart defects in most of the embryos by E14.5. These results highlight the importance of specific integrins in developmental angiogenesis but also that compensation mechanisms by other integrins exist in order to complete angiogenesis. These mechanisms are not only observed during development but also for example in adult hypoxic brain endothelial cells [41]. The compensation mechanisms may play crucial role and should be taken into consideration when analyzing the results of integrin targeting therapy.

In addition to its direct role in angiogenesis, integrin $\alpha 5\beta 1$ also regulates angiogenic signals by binding with different partner such as endostatin [42], VEGFR-1 [43], Angiopoietin-2 and Tie-2 [44]. Interestingly, mature vessels present very low level of $\alpha 5$ integrin subunit (with the exception of hepatic sinusoid and high-endothelial venules in lymph nodes) whereas tumor vasculature or neovessels in the cornea express high level of $\alpha 5$ [45–48]. Injection of a specific monoclonal anti- $\alpha 5$ antibody in several murine cancer models shows that $\alpha 5$ integrin subunit is expressed on the luminal side of the tumor vasculature and thereby directly accessible for potential anti- $\alpha 5$ agents [49]. Integrin subunit $\alpha 5$ expression in endothelial cells is regulated by several angiogenic factors such as FGF, TNF α or IL8, but not VEGF [45]. In turn, activation of $\alpha 5\beta 1$ on endothelial cells by attachment to fibronectin results in the transcription of a gene repertoire related to angiogenesis (HB-EGF, IL8, CXCL1), adhesion (VCAM, E-selectin), signal transduction (RICK, NF κ b) and coagulation (TF) [50]. Once expressed, $\alpha 5$ integrin subunit promotes survival signals in angiogenic endothelial cells and blocks apoptotic signals independently of attachment to matrix *in vitro* and *in vivo* [51]. Therefore, blocking $\alpha 5$ integrin subunit with a small peptide or an antibody results in anti-angiogenic effects and reduced tumor growth by integrin-mediated death pathway [45,52,53]. Due to its unambiguous role in angiogenesis, $\alpha 5\beta 1$ integrin has become a target for anti-angiogenesis therapy.

4. Integrin $\alpha 5\beta 1$ in Solid Tumors

4.1. Colon Tumors

The controversy about $\alpha 5\beta 1$ integrin as a tumor suppressor rather than a protumoral integrin mainly arose from data obtained in a colon cancer cell line, HT29. Studies showed that *de novo* expression of $\alpha 5$ integrin subunit in HT29 cells results in cell growth arrest *in vitro* and decreased tumorigenicity *in vivo*. Cell growth arrest was reversed by ligation of $\alpha 5\beta 1$ integrin to fibronectin [54]. Interestingly, $\alpha 5\beta 1$ -expressing HT29 cells were shown to resist to serum deprivation-induced apoptosis [55]. The tumor suppressive function of $\alpha 5\beta 1$ integrin in HT29 cells was confirmed in another study [56] and a strong inhibitory action of this integrin on lung colonization and metastasis was also reported [57]. These results were challenged when subgroups of colon cancer cell lines were examined according to their differentiation status [58]. It was shown that integrin $\alpha 5\beta 1$ level was increased in the poorly differentiated group in relationship with an increased capacity to form tumors in nude mice [59]. In

accordance with these results, three well-established colon cancer cell lines, KM20, KM12C and KML4A, treated with an anti- $\alpha 5$ integrin inhibitory antibody, increased their apoptosis rate [60]. It was recently found that 19% of colon carcinoma, over 94 tumors examined, expressed $\alpha 5\beta 1$ integrin at the protein level and in these tumors the labeling concerned only a fraction of neoplastic epithelial cells [61]. Interestingly, acquisition of $\alpha 5\beta 1$ integrin was correlated with ADAM-15 down-regulation and poor prognosis [61]. In line with this, hypoxia was shown to increase $\alpha 5$ integrin subunit at the mRNA level and this increase was more prominent in Duke stage C and D patients than in Duke stage A and B patients suggesting that the transcription increases along with the progression of colon cancer [62]. Upregulation of $\alpha 5$ integrin subunit gene transcription in colon cancer cells is under the control of PTHrP [63] or ZEB2 [64] and leads to an upregulation of cell invasion during epithelial-mesenchymal transition. Activation of $\alpha 5\beta 1$ integrin and corresponding signaling pathways by P-selectin and the human carcinoembryonic antigen (CEA) was also reported in colon carcinoma cells [65,66]. Suppression of $\alpha 5\beta 1$ integrin activity by lunasin, a peptide isolated from soybean and having an RGD motif, potentiates the effect of oxaliplatin thus preventing outgrowth of colon cancer metastasis [67].

4.2. Ovarian Tumors

Peritoneal dissemination is an important step in ovarian cancer progression to invasion and metastasis. It was first reported that fibronectin secreted by peritoneal tissue activates $\alpha 5\beta 1$ integrin on ovarian cancer cells to stimulate their invasiveness through an increase of MMP-9 activity [68]. $\alpha 5\beta 1$ integrin regulates the formation of ovarian carcinoma multicellular spheroids, an *in vitro* model of micrometastasis [69], and partially mediates adhesion to mesothelial cell monolayer of patient-derived ascites spheroids [70]. Many human ovarian cancer cell lines express $\alpha 5\beta 1$ integrin and their binding to mouse peritoneal wall preparation was impaired specifically by anti $\alpha 5\beta 1$ integrin antibodies or endostatin which is a ligand for $\alpha 5\beta 1$ integrin [71,72]. Kallikrein-related peptidases (KLK) are serine proteases often upregulated in ovarian carcinoma. KLK7 overexpression correlates with formation of large compact spheroids, chemoresistance and poor outcome in clinical settings. Interestingly enhanced expression of KLK7 in ovarian cancer cell lines and clinical samples was associated with enhanced expression of $\alpha 5\beta 1$ integrin [73] suggesting that $\alpha 5\beta 1$ integrin participates to the poor outcome of patients. The hypothesis of $\alpha 5\beta 1$ integrin as a prognostic marker in ovarian tumors is confirmed by other data including large cohorts of patients [74,75]. In one of this study [74], $\alpha 5\beta 1$ integrin expression was inversely correlated with E-cadherin expression and was shown to be implicated in adhesion of tumor cells to the peritoneal cavity and metastasis. Inhibition of $\alpha 5\beta 1$ integrin by specific antibodies led to the suppression of intra-peritoneal tumor spread and increased survival in two xenograft models of ovarian cancer. In fact fibronectin/ $\alpha 5\beta 1$ integrin interaction on ovarian cancer cells activates the oncogene cMet and provides key mitogenic-signalling pathways to the cells [76]. Adrenomedullin also upregulates $\alpha 5\beta 1$ integrin in ovarian tumors and patients with high adrenomedullin expression showed a higher incidence of metastasis and poor outcomes, indirectly further suggesting a role of $\alpha 5\beta 1$ integrin in the aggressiveness of ovarian tumors [77]. An overview of integrin inhibitors as therapeutic agents for ovarian cancer has been published very recently [78].

4.3. Breast Tumors

Similarly to what was shown in colon cancer cells, the first data concerning $\alpha 5\beta 1$ integrin in breast tumor cells were in favor of its tumor suppressive effect. It was reported that treatment of the highly invasive breast carcinoma cell line MDA-MB-435 (which has been further classified as a melanoma cell line) with Maspin suppressed their invasive phenotype through an increased expression of $\alpha 5\beta 1$ integrin at the mRNA and protein level [79]. Subsequent data however challenged this view as they demonstrated a proinvasive role of $\alpha 5\beta 1$ integrin in breast cancer cells [80–82]. The oncogene ERBB2, strongly associated with metastatic disease and poor prognosis, drives the transcriptional upregulation of $\alpha 5\beta 1$ integrin in mammary adenocarcinoma promoting tumor cell survival under adverse conditions and invasive capacity [80,83]. In a subset of breast cancers, overexpression of Steroid Receptor Coactivator-1 (SRC-1) was associated with an upregulation of $\alpha 5\beta 1$ integrin and promotion of $\alpha 5\beta 1$ integrin-dependent cell adhesion and migration [84]. Inverse relationship between $\alpha 5\beta 1$ integrin expression and tumor suppressors expression such as nischarin [85], metastasis suppressors such as Nm23 [86] or epithelial cell-cell adhesion marker such as E-cadherin [87] were reported and associated with impact on breast cell tumorigenic potential. Loss of E-cadherin was also achieved through stimulation of breast cancer cells by angiopoietin-2 which stimulated cell migration through an $\alpha 5\beta 1$ integrin-dependent way [88]. Data also showed that $\alpha 5\beta 1$ integrin controls invasion of breast cancer cells by modulation of MMP-1 [81] and MMP-2 collagenase activity [89]. $\alpha 5$ integrin subunit mRNA was weakly expressed in normal tissues and more strongly expressed in breast cancer specimens [90] and elevated $\alpha 5$ integrin subunit gene expression was associated with decreased long term survival in one cohort of patients with breast cancer [91] but not in two other cohorts [92]. Interestingly, while $\alpha 5$ integrin subunit was proposed to be positively involved in lung metastasis of breast tumors in humans [85], the opposite effect was described for mouse breast tumor cells [93]. Finally, radiotherapy was shown to increase $\alpha 5\beta 1$ integrin expression level in 3D culture breast tumor cells and combined cell treatment with ionizing radiation and antagonists of $\alpha 5\beta 1$ integrin triggered apoptosis [91].

4.4. Lung Tumors

Tobacco is the major risk factor for lung tumors. The main tobacco alkaloid nicotine stimulates lung cancer cell proliferation by the induction of fibronectin that led to activation of the $\alpha 5\beta 1$ integrin. In non small cell lung cancer, $\alpha 5\beta 1$ integrin overexpression at the mRNA [94,95] or protein [96,97] level was negatively associated with patient survival. Interestingly, $\alpha 5\beta 1$ integrin expression could differentiate between adenocarcinoma and squamous cell carcinoma of the lung [94]. $\alpha 5\beta 1$ integrin expression was more frequent in tumors with lymph node metastasis than in those without metastasis [96]. Fibronectin- $\alpha 5\beta 1$ integrin signaling has been studied and implicated in lung cancer progression [98–100] and reviewed in [101]. The PI3K/AKT/mTOR pathway is a key mediator of fibronectin-integrin effects on proliferation [99]. Extracellular matrix proteins including fibronectin were shown to protect lung cancer cells from apoptosis through $\beta 1$ integrin activation [102–105] thus explaining drug resistance of lung cancer cells.

4.5. Glioma

The $\alpha 5\beta 1$ integrin is expressed at significantly higher level in glioblastoma (the most aggressive glioma) than in adjacent normal brain tissue suggesting that it might play a role in the development or the progression of glioma [106]. $\alpha 5\beta 1$ integrin is commonly expressed in a perinecrotic or perivascular pattern in glioblastoma [107]. $\alpha 5$ integrin subunit mRNA level is under the control of the transcription factor ETS-1 and its expression is related to the grade of glioma, with the highest expression in glioblastoma [108]. We [109,110] and others [111] confirmed recently these data in larger cohorts of patients and showed that a high expression of $\alpha 5\beta 1$ integrin is associated to a worse prognosis for patients with glioma. We also demonstrated that $\alpha 5\beta 1$ integrin expression is under the negative control of caveolin-1 and positive control of TGF β R in a subset of glioma tumors [110,112]. By the use of specific non peptidic antagonists of $\alpha 5\beta 1$ integrin, its role in proliferation, migration, invasion and resistance to chemotherapy was highlighted in different glioma cell lines [109,113,114]. Interaction of MMP-2 with $\alpha 5\beta 1$ integrin was shown to regulate the IL-6/STAT3 survival signaling in glioma [115]. The expression of the DNA repair protein, O6-Methylguanine-DNA Methyltransferase (MGMT), was inversely related to invasion capacity of glioma and to $\alpha 5\beta 1$ integrin expression [116].

4.6. Melanoma

Malignant melanoma has a high metastatic potential. A role of $\alpha 5\beta 1$ integrin in promoting melanoma metastasis through an increase in cell adhesion to fibronectin and protection against apoptosis was reported [117]. Recently, evidence that $\alpha 5\beta 1$ integrin has a crucial role in melanoma metastasis confirmed this hypothesis. It was shown that $\alpha 5$ integrin subunit up-regulation was under the control of survivin [118] or controlled by the interaction between caveolin 1 and Rho-GTPases [119]. Curiously, the specific uveal melanoma seems to be one of the cases where $\alpha 5\beta 1$ integrin expression negatively impacts on tumorigenicity. High aggressiveness of uveal melanoma cells is dependent on the loss of $\alpha 5\beta 1$ integrin at the cell surface [120–122]. However, restoration of $\alpha 5\beta 1$ integrin expression in high tumorigenic cells increased the cell resistance to stress *in vitro* and growth properties *in vivo* [121] which appears somewhat paradoxical. It has been proposed that the effect of $\alpha 5\beta 1$ integrin on cell tumorigenicity depends on the endogenous expression of fibronectin by the tumoral cells.

5. Integrin $\alpha 5\beta 1$ Antagonists

The search for specific $\alpha 5\beta 1$ integrin antagonists has increased these last years. They are developed to understand the integrin pathophysiological behaviour in preclinical studies on endothelial and tumoral cells but also as therapeutic agents in the clinic [2]. As $\alpha 5\beta 1$ integrin has been largely described as an unambiguous pro-angiogenic integrin, these antagonists are generally presented as potential anti-angiogenic agents. Three main classes of antagonists are described at the time, specific antibodies, small peptides or small non peptidic RGD-like molecules.

5.1. Antibodies

An $\alpha 5\beta 1$ function-blocking murine antibody, IIA1, was used in preclinical studies [74,91]. It was able to inhibit *in vitro* invasion of ovarian tumor cells into Matrigel and tumor cell adhesion to mesothelial cells; it decreased the number and the size of intra-abdominal metastases and increased the survival of mice [74]. This inhibitory antibody also induced apoptosis of breast cancer cells in 3D culture conditions [91]. A chimeric humanised version of IIA1 antibody was generated, named volociximab (developed first by PDL Biopharma, Fremont, CA, USA), with similar affinity for $\alpha 5\beta 1$ integrin and similar activity for blocking integrin adhesion to fibronectin than IIA1 [123]. Volociximab is a potent inhibitor of *in vitro* model of angiogenesis by inducing apoptosis of actively proliferating but not resting endothelial cells. It reduced vessel density and tumor growth in carcinoma xenografted in rabbits [123,124]. Results of volociximab in clinical assays have been reviewed recently in [125]. Volociximab has been shown to be safe and tolerable in phase I studies [126,127] in patients with different solid tumors. Reported adverse effects included constitutional symptoms, gastrointestinal symptoms, headache, edema and hypertension. Although $\alpha 5\beta 1$ integrin is expressed on normal blood monocytes, no clinically apparent infectious complications were observed. A phase II clinical trial has shown that in patients with platinum-resistant advanced epithelial ovarian or primary peritoneal cancer, weekly monotherapy with volociximab was well tolerated but without efficacy on these particular population of patients [128]. In patients with refractory or relapsed metastatic clear cell renal carcinoma, volociximab led to stable disease in 80% of patients [129].

A dual functional monoclonal antibody, PF-04605412, has been developed by Pfizer. This antibody targets $\alpha 5\beta 1$ integrin and was engineered to elicit potent antibody-dependent cellular toxicity [130]. Preclinical studies showed that PF-04605412 potently inhibited $\alpha 5\beta 1$ integrin mediated intracellular signalling, cell adhesion, migration and angiogenesis. In animal studies, it displayed robust anti-tumor efficacy correlated with $\alpha 5$ integrin subunit expression, macrophages and natural killer cells infiltration [130]. A clinical trial phase I is currently underway in solid tumors refractory to available therapies.

5.2. RGD-like Molecules

The RGD motif of fibronectin is recognised by at least three main integrins: $\alpha 5\beta 1$, $\alpha v\beta 3$ and $\alpha IIb\beta 3$. The challenge of these last ten years has been to design antagonists with enhanced selectivity for each of these integrins.

The first selective non peptidic antagonist for $\alpha 5\beta 1$ integrin was SJ749 (compound 20 in [131]). SJ749 blocked efficiently $\alpha 5$ integrin-expressing HT29 cell adhesion to fibronectin and not to other ECM ligand. It also blocked $\alpha 5\beta 1$ integrin function in chick embryo and murine models of angiogenesis acting as a potent inhibitor of tumor growth and tumor-induced angiogenesis [45]. We described that SJ749 potently inhibited the proliferation of glioma cell lines dependently of $\alpha 5\beta 1$ integrin expression level [112,114] and that SJ749 sensitized glioma cells to chemotherapy by modulating the p53 pathway [113].

SJ749 was used in docking experiments to build a 3D model of the $\alpha 5\beta 1$ integrin with the $\alpha v\beta 3$ integrin crystal structure as a model [132]. Based on the characteristics of SJ749 binding site and SAR

analysis, analogs of SJ749 [132] or original compounds [133–135] were designed by the group of H. Kessler (München, Germany) and tested for their integrin affinities. Compounds with high affinity and selectivity for $\alpha 5\beta 1$ integrin were found by these strategies. Few data concerning the biological activities of such compounds are available to date. We evaluated the effects of one of these compounds, K34c, on glioma cell lines. We demonstrated that K34c affected the survival of glioma cells as well as their resistance to chemotherapies [110,113].

New selective small non peptidic $\alpha 5\beta 1$ integrin antagonists were described by Jerini AG (Berlin, Germany) [136]. Compounds were mainly tested in pathological models of neovascularization where $\alpha 5\beta 1$ integrin plays a crucial role [137–141]. One of them, JSM6427, was shown to attenuate glioma growth [141]. Interestingly, new orally available $\alpha 5\beta 1$ integrin antagonists were described recently by this pharmaceutical group [142,143]. Other small non peptidic molecules were synthesized by AstraZeneca and showed some selectivity for $\alpha 5\beta 1$ integrin compared to $\alpha v\beta 3$ integrin [144,145].

5.3. Non RGD-like Peptides

Sequences outside of the RGD site are required to allow full adhesion of $\alpha 5\beta 1$ integrin to fibronectin. Of particular interest is the sequence Pro-His-Ser-Arg-Asn (PHSRN) in the 9th type III repeat of fibronectin also called the “synergy site”. PHSRN peptide induced invasion of prostate tumor cells by inducing MMP-1 [146,147] and stimulation of angiogenesis [148] which was inhibited by the competitive inhibitor PHSCN peptide. The acetylated amidated PHSCN peptide was even more potent than PHSCN peptide [146], and was developed by Attenuon LLC (San Diego, CA, USA) under the name ATN-161. ATN-161 treatment blocks prostate tumor recurrence, metastasis and micrometastasis [149], reduces colorectal liver metastasis and improves survival when given in addition with chemotherapy [150], and blocks breast cancer growth and metastasis [151] in preclinical mouse models. Targeting $\alpha 5\beta 1$ integrin with ATN-161 in combination with radiotherapy enhanced apoptosis of breast cancer cells grown in 3D culture [91]. ATN-161 proved also efficient to block choroidal neovascularisation [152]. Phase I trial of ATN-161 indicated that it was well tolerated in patients with solid tumors and that one third of patients manifested prolonged stable disease. No side effects emerged or became worse with continued chronic dosing of ATN-161 [153]. Recently, PHSCN dendrimers were synthesized and shown to be more potent than the initial peptide for inhibiting $\alpha 5\beta 1$ integrin-mediated MMP-1 secretion *in vitro* and for inhibiting human prostate cancer cell invasion, extravasion and lung metastasis *in vivo* [154]. Similar results were reported on human breast cancer cells [155].

6. Conclusions

The critical role of $\alpha 5\beta 1$ integrin in physiological angiogenesis and development has been recognized for over two decades. More recent are the data implicating $\alpha 5\beta 1$ integrin in pathophysiological/tumoral neoangiogenesis. Even more recently, its role as a prognostic and diagnostic marker has been highlighted in several solid tumors. The relationship between high expression of $\alpha 5\beta 1$ integrin in subpopulation of patients with solid tumor and a poor prognosis for these patients suggest its implication in resistance to conventional therapies. As shown above, $\alpha 5\beta 1$ integrin is implicated in different aspects of tumor progression and appears particularly overexpressed in the most aggressive tumor grades. Ways to modulate positively the $\alpha 5\beta 1$ integrin expression also

appear multiple and certainly tissue dependent. Its participation in tumor angiogenesis and tumoral cell migration and adhesion to metastasis niches as well as its effects on therapy resistance make it a pertinent therapeutic target for the future. Several antagonists are being tested with some already reaching the clinic. Targeting $\alpha 5\beta 1$ integrin appeared safe for the patients in the few clinical trials reported so far. To date, efforts have not focused on $\alpha 5\beta 1$ integrin antagonists but data summarized here support the notion that they will play an increasing role in human therapy. The recent elucidation of the crystal structure of $\alpha 5\beta 1$ integrin ectodomain will certainly help to define more potent and specific antagonists. The goal for the future will be to define clear molecular biomarkers to support the proposition of subpopulations of patients potentially sensitive to a targeted therapy against $\alpha 5\beta 1$ integrin.

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Conflict of Interests

The authors declare no conflict of interest.

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