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Biological relevance of RGD-integrin subtype-specific ligands in cancer

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ABSTRACT

Integrins are heterodimeric transmembrane proteins able to connect the cells with the micro-environment. They represent a family of receptors involved in almost all the hallmarks of cancer. Integrins recognizing the Arg-Gly-Asp (RGD) peptide in their natural extracellular-matrix ligands, have been particularly investigated as tumoral therapeutic targets. In the last 30 years, intense research was dedicated to design specific RGD-like ligands able to discriminate selectively the different RGD-recognizing integrins. Efforts of chemists led to the proposition of modified peptide or peptidomimetic libraries to be used for tumor targeting and/or for tumor imaging. Here we review, from the biological point of view, the rational underlying the need to clearly delineate each RGD-integrin subtype by selective tools. We describe the complex roles of RGD-integrins (mainly the most studied αvβ3 and α5β1 integrins) in tumors, the steps towards selective ligands and the current usefulness of such ligands. Although the impact of integrins in cancer is well acknowledged, the biological characteristics of each integrin subtype in a specific tumor are far from being completely

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resolved. Selective ligands may help to reconsider integrins as therapeutic targets in specific clinical settings.

1 INTRODUCTION

Integrins are heterodimeric transmembrane proteins recognized first as adhesion molecules on specific extracellular matrix (ECM) components. They were further assigned to a true receptor family as their ability to initiate intracellular signaling pathways was largely emphasized. In the hallmarks of cancer as defined by Hanahan and Weinberg in 2000 [1], integrins were acknowledged as actors of self-sufficiency growth signaling, as regulators of anti-apoptotic signaling, angiogenesis and adaptation to new environments during invasion and metastasis of tumor cells. New hallmarks were added ten years later [2],in particular, the contribution of the tumor microenvironment (TME) to tumorigenesis. The TME does not only contain ECM but also recruits immune and stromal cells able to facilitate tumor growth. Changes in the repertoire and expression level of integrins in these non tumoral cells were described to support aggressive tumoral phenotypes [3]. Integrins were recognized early as therapeutic targets in cancers [4–6]. The most studied integrins in oncology belong to the RGD-integrin subfamily with αvβ3/β5 and α5β1 integrins in the spotlight for the two last decades [7]. The RGD (Arg-Gly-Asp) peptidic motif is found in ECM proteins (fibronectin, vitronectin, osteopontin, thrombospondin among others) and represents a selective binding site for integrins to ECM. Since activation of integrins by adhesion to their ECM ligands was shown to be essential to non-tumoral adherent cell survival [8,9], similar effect was expected for tumoral cells. Disrupting the interactions between integrins and ECM through RGD-like compounds appeared therefore a valuable strategy to trigger tumor cell apoptosis. Research in the design and characterization of integrin antagonists as cancer drug candidates has expanded rapidly [10]. Cilengitide, a cyclic RGD peptide, was the first αvβ3/β5 integrin antagonist that reached the clinic [11]. Despite numerous preclinical encouraging data, cilengitide failed to improve glioblastoma patient's survival in phase II/III randomized clinical trials [12,13]. No better outcomes were obtained with Volociximab or MINT1526A, both α5β1 integrin inhibitory antibodies, in several clinical settings [14,15]. These disappointing results impeded considerably research on RGD-integrins as therapeutic targets in oncology. However, recent reviews on integrins and cancer emphasize the need to reconsider the topic [6,7,16–20]. In this review we will focus mainly on RGD-integrins αvβ3 and α5β1 as most ligands have been designed for them. We will support the idea that integrins should still be considered as targets for anti-cancer therapies. In addition, ligands specific for definite subtypes of integrins will help in understanding how and where integrins must be targeted and will shed more light on the complexity of the topic.

2 RGD-integrins in cancer

2.1. Integrins in focal adhesion and cell migration

Common rules of integrins have been extensively reviewed elsewhere (for a recent review see [21]). In human, 18 α and 8 β subunits can combine to form heterodimeric transmembrane

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proteins with specific ligand recognition properties. They share an architecture comprising an extracellular domain, a transmembrane region, and a generally short intracellular part. If specific adhesion to ligands lies in the composition of the αβ extracellular head, integrins signal inside the cells by structural changes and recruitment of cytoplasmic proteins in complexes named adhesomes [22]. Such complexes contain about 2000 proteins among which a highly dynamic "consensus adhesome" of 60 proteins may control integrin dependent cell adhesion and signaling [23]. Mechanical forces can influence the assembly/disassembly of these macro-molecular structures adding a further level of complexity [24]. Recent work pointed that β3 integrins were more strongly bound to their substrate in adhesion sites submitted to high tension than β1 integrins [25]. The dynamic nanoscale organization of integrins and their regulators within focal adhesion points was shown to differ between β_3- and β1-integrins in the control of signaling during cellular functions [26,27]. Although both integrins recognize fibronectin, striking differences between α5β1 and αvβ3 integrin signaling pathways have been described in normal/tumoral cells. Most data addressed the question of focal adhesion point maturation and cell migration. αvβ3 integrins were associated with persistent migration through activation of Rac-mDia1, whereas α5β1 integrins were linked to RhoA-Rock-MyoDII pathway and random migration [28]. The role of both fibronectin-binding integrin classes can begin with an initial competition followed by a cooperative crosstalk [29]. Alternatively, blocking αvβ3 integrin may activate α5β1 integrin recycling back to membrane and increase cell migration [30]. Distinct mechanisms and/or efficient crosstalk between both integrins have emerged over the years [31]. New insights on focal adhesion maturation have been made possible using ligands specifically designed to target either αvβ3 or α5β1 integrins. It was shown that focal adhesion maturation on α5β1 integrin-selective substrates is dependent on αvβ3 integrin recruitment [32–34]. Most of the above described knowledges were obtained in non-tumoral cells and translation to cancer cells may be even more decisive for their functional behavior as the expression of integrins and the ratio between αvβ3 and α5β1 integrin levels are specifically altered in tumoral tissues as is the case for the expression of their common ECM ligand fibronectin [35].

2.2. Integrins in hallmarks of cancer

Thirty years ago integrin αvβ3 emerged as a marker of tumoral neo-angiogenesis which was inhibited by specific antagonists such as cilengitide in preclinical models [36–38]. These results were challenged as enhanced pathological angiogenesis and increased primary tumor growth were observed in mice lacking β3 or β3/β5 integrins [39]. Specific overexpression of Integrin β3 in tumor cells suppressed tumor growth in a human model of gliomagenesis [40]. Additionally, it was shown that nanomolar concentrations of RGD-mimetic αvβ3/αvβ5 inhibitors can enhance the growth of tumors in vivo by promoting VEGF-mediated angiogenesis [41]. These data may explain in part why the therapies with these inhibitors have failed in humans [12,13]. Other integrins participate in angiogenesis [42] and the proangiogenic function of α5β1 integrin has been clearly demonstrated [43–45]. It was shown that reduced expression of the α5 subunit is associated with reduced blood vessel formation and tumor growth[46] and that α5β1 integrin was overexpressed in tumoral vessels [47]. It will be essential to reconsider and to revisit fundamental biology underlying how these integrins coordinate angiogenesis to successfully target them [48].

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Beside their role in angiogenesis, both integrins are overexpressed in many tumoral cells and thought to participate in enhanced survival and resistance to therapies [7,20,49–52]. This overexpression is mainly characterized at the mRNA level but less data exist at the protein level in cohorts of patients. The large transcriptomic databanks available nowadays for different tumor types help not only to define the inter-patient heterogeneity but also to correlate specific gene overexpression in tumor subtypes to patient survival. They have been used for high grade brain tumors (glioblastoma, GBM) for example [53]. In these tumors, α5 and β1 integrin mRNAs are overexpressed in GBM versus normal brain but even more in the GBM mesenchymal subgroup [54]; it was less subgroup dependent for αv and β3 integrin mRNA [55]. α5 and β1 genes are included in the GBM mesenchymal signature so that they represent biomarkers of this aggressive subtype. Interestingly α5 integrin is also overexpressed in subclones of pediatric brain tumors [56]. Extensive evaluation and comparison of integrin gene expression in different tumors and tumor subtypes would be a first step towards efficient anti-integrin therapeutic strategies. Even if determination of integrin proteins in cell lines is a prerequisite to characterize functional implication in vitro, the missing link is however the characterization of integrins at the protein level in clinical studies. Few data on small- to medium-sized patient cohorts are available in the literature. Immunohistochemical analysis of αv or β3 integrin protein expression in such cohorts were recently reported in osteosarcoma [57], hepatocellular carcinoma [58], leukemia [59] and the relationships with patient survival established. A retrospective immunohistochemical analysis of αvβ3, αvβ5 or αvβ8 integrins in the glioblastoma patient cohorts of cilengitide clinical trials was done. It revealed that only αvβ3 integrin expression in tumoral cells may predict benefit from cilengitide inhibition in a subset of patients with glioblastoma lacking *MGMT* promoter methylation [60]. Similarly, high levels of α5 or β1 integrin protein expression corresponded to worse survival in oral carcinoma [61], triple negative breast cancer [62], pancreatic cancer [63], osteosarcoma [64] and colorectal cancer [65]. In the last period, data concerning integrin expressions and functions in non-tumoral cells in the microenvironment of tumors have been increasingly reported. Roles of integrins in Cancer Associated Fibroblasts (CAF) have been reviewed recently [66]. Engineered mice models allowed deletion of integrin gene in specific population of cells. It was shown that acute deletion of β3 integrin in endothelial cells transiently diminished tumor growth and angiogenesis but long term deletion was ineffective [67]. Specific deletion of this gene in myeloid cells resulted in enhanced tumor growth [68] and increase of M2 macrophages at the tumor site thus promoting pro-tumoral functions in the microenvironment [69]. In another work, it was shown that tumor- associated -macrophages (TAM) accumulation correlated with tumor cell expression of αvβ3. This characteristic was used to design an αvβ3 antibody-dependent cellular cytotoxicity (ADCC) against tumoral cells [70]. Two recent works underlined the expression of α5 integrin in the stroma of pancreatic [71] and colorectal tumors [72]. In the former study, inhibition of this integrin in pancreatic stellate cells attenuates tumorigenicity and potentiates efficacy of chemotherapy; in the latter, integrin depletion reduced the ability of CAF to promote cancer cell migration presumably by the down regulation of fibronectin.

All the non-exhaustive recent findings described in the first part of this review suggest that we need to better understand the roles of RGD-integrins in tumors. Encouraging preclinical discoveries with integrin inhibitors did not translate to clinical successes due to the complexity

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of integrin biology. We will have to consider not only integrins in tumoral cells and vessels but also in the tumor microenvironment including the immune system (Figure 1). For this point, preclinical modeling of the clinical reality remains a challenge. The overexpression of some integrins in treatment-resistant cells or at metastasic sites must be thoroughly characterized. We will have to refine the positive or negative crosstalk between different integrins and their relationships in specific tumor area or in specific subset of patients. We need to compare different integrin subtypes more systematically to address their respective contribution to tumorigenicity and optimize the use of specific inhibitors. The availability of specific and selective integrin ligands is of great importance to go further on.

3 RGD-integrin ligands

Three main classes of integrin ligands have been proposed: antibodies, RGD-derived peptides and RGD-mimetic small molecules (Figures 2 and 3). More recently, aptamers (short nucleic acid sequences) have been included in this list [73,74]. While antibodies may be highly integrin subtype-specific by nature, RGD-derived ligands may recognize all RGD-binding integrins. Efforts towards achieving high selectivity of RGD-integrin ligands were actively made during the last two decades with, for example, the pioneer works of H. Kessler's group. Although recognizing the same tripeptide sequence in their natural ligands, similarities and differences between RGD-integrins have been highlighted based on their crystal structures. The structures of the extracellular fragment of integrins αvβ3, αIIbβ3 and α5β1 were respectively resolved in 2001 [75,76], 2004 [77] and 2012 [78] helping to understand how ligands fit in the integrin binding sites.

3.1. Specificity, selectivity, and activity of integrin ligands.

Until today, integrin ligands were mainly tested for their capacity to bind integrins and to disrupt their interactions with their preferred natural ligands, using as a readout the role of integrin as adhesion proteins. For the evaluation of large libraries of compounds, tests must be rapid and reliable for lead characterization and optimization. They are based either on cell adhesion assays or on purified soluble integrins adhesion assays on specific ECM substrates. Results (expressed as IC50 values) obtained by both approaches are strongly variable depending on several parameters. Rational design of such tests appears crucial to compare ligands for one integrin or one ligand for different integrins. Recent works give insights in this topic [79,80]. Concerning activity, the notion of integrin antagonism versus agonism is fairly taken into account in the first steps of ligand selection. This point becomes critical in the field of oncology where disruption of cell adherence does not automatically relate to cell death. Moreover, RGD-like compounds may behave as true agonists mimicking the ECM natural ligand-dependent activation of integrins and subsequent pro-tumoral signaling pathways [41]. Very interestingly, recent developments on integrin pure antagonists have been published supporting a new area in integrin ligand research [81–84]. Based on electron microscopy, X-ray chrystallography and receptor priming studies, it was shown that these new classes of integrin antagonists do not induce the integrin conformational changes associated with activation.

3.2 Antibodies

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Specific antibodies against RGD-integrins contributed to study integrin activation-state regulation, integrin biology and integrin-based therapeutics. In contrast with peptides or peptide-mimetics acting essentially at the RGD binding domain, they cover a large set of epitopes localized in different structural parts of the α or β integrin subunits [85]. They can be classified into three main groups: stimulatory or activation-specific, inhibitory and non-functional antibodies as largely described by the group of M. Humphries [86–88]. It must be emphasized here that integrin conformations are known to be variable in relation to their functional status which addressed mainly their capacity to adhere to ECM ligands. The bent, the extended closed and the extended open global conformations are likely to be shared by the majority of integrins. Using electron microscopy and biophysical thermodynamic analysis on α5β1 integrin and with the help of specific antibodies, it was shown that only the extended-open conformation mediates adhesion to fibronectin and that intrinsic affinity depends on specific integrin conformational states [89,90]. Antibodies against α5β1 and αvβ3 integrins are thus widely used in preclinical studies to depict fundamental cues, label or inhibit these integrins. Among inhibitory antibodies, some reached early phases clinical trials (volociximab for α5β1 integrin and Intratumumab/CNTO95 or abituzumab for αvβx integrin) [91,92]. But despite being well tolerated, they failed to progress to phase II/III trials.

3.3. RGD-derived peptides

Cilengitide, a cyclic pentapeptide, developed twenty years ago by Horst Kessler and his group, became the prototype of RGD-derived peptides with enhanced selectivity towards αvβ3 integrin. The story of cilengitide is extensively described in [93] explaining the different steps of development from a linear, flexible and non-selective RGD peptide to a cyclic, rigid and selective one. In the search for peptides with increased selectivity either towards αvβ3 or α5β1 integrins, multiple strategies have been explored using isoDGR motifs [94,95], cyclic aza-peptides [96] or di-N- methylation of cilengitide [97]. Cilengitide remains the reference compound in the field and integrin binding characteristics of new integrin ligands are often compared to it. Other groups worked around the cyclic pentapeptide structure. For example, cyclic RGD pseudopentapeptide incorporating bicyclic lactams were described [98] and further optimized leading to potent antagonists of αvβ3/β5 integrins [98–101]. Series of cyclo-octapeptides including RGD have also been described. Based on One-bead One-Compound combinatorial library technology, the lead compound LXW7 was discovered [102] and optimized as LXW64 and LXZ2 [103,104]. These compounds proved able to selectively label tumoral cells expressing αvβ3 in vitro and in vivo. Recently, a novel specific integrin αvβ3 targeting linear pentapeptide, RWrNK, was described [105]. Its development used a structure-based pharmacophore method integrated with molecular docking. Bifunctional diketopiperazines were introduced into cyclic peptidomimetics containing the RGD sequence (DKP-RGD) leading to compounds specific for αvβ3/β5 integrins [106]. Interestingly, these compounds exhibited binding affinities towards α5β1 integrin but always lower than for αvβ3 integrin [107]. Thus using several approaches, numerous peptidic ligands specific/ selective for αvβ3/β5 integrins have been made available unlike those for α5β1 integrin. The isoDGR peptide library [95] was screened to confer to the small lead pentapeptide selectivity towards the fibronectin- binding integrins α5β1 and αvβ6 [94]. Through sequential N-methylation, the biselective c(phg-*iso*DGR-k) was converted to c(phg-isoDGR-(NMe)k) which appeared as a selective α5β1 integrin ligand

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[108]. It should be noted that other peptides highly selective for α5β1 integrin have also been proposed without an RGD sequence. The first example is the Cys-Arg-Arg-Glu-Thr-Ala-Trp-Ala-Cys (CRRETAWAC) peptide originally discovered in 1993 from the screening of a phage-display library in which heptapeptides were flanked by cysteine residues, thus making the inserts potentially cyclic [109]. Further investigations showed that residues of the α5 subunit involved in recognition of RRETAWA are predicted to lie close to those involved in RGD binding but do not completely overlap [110]. The second example is ATN161, a capped five amino-acid peptide derived from the synergy region PHSRN of fibronectin (which contributes to high affinity recognition of fibronectin by α5β1 integrin). It was shown to be very selective for α5β1 integrin over αvβ3/β5/β6/β8 integrins in an ELISA test using soluble purified integrins [79]. Compared to other integrin ligands, ATN-161 has the particularity of not competing with the fibronectin binding and thus is unable to detach the cells from the matrix. It was included in two clinical trials (for advanced renal cancer and recurrent glioblastoma) but no results were posted to date.

Panels of selective RGD-derived peptides are available. Few of them have been studied as true antagonists of integrin signaling pathways and pro-tumoral effects. However, knowledge on the mode of binding of RGD-containing peptidomimetics has largely increased with the advent of computer-assisted docking studies in the crystal structure of the integrin binding sites. This helped and will further improve the design of new ligands.

3.4. Small molecules as RGD-mimetics.

In parallel with the design of RGD-derived peptides, search for RGD-mimetics, led to the discovery of selective more stable and bioavailable small molecules as integrin ligands. The first goal of research was to obtain selectivity for αvβ3 versus αIIbβ3 integrins. This latter RGD-integrin is involved in platelets regulation and undesirable antagonist side effects had to be avoided. The second goal was to evaluate the possibility of distinguishing the αvβ3 integrin from the α5β1 in order to have highly selective ligands even though their RGD binding site are highly homologous. Both goals were achieved by pharmaceutical and academic groups. A review recapitulated the αvβ3 antagonists available in 2000 [111]. The field has progressed and began to focus also on α5β1 integrin. The initial series of αvβ3 integrin ligands were generally also recognized by α5β1 integrin. A series of nonpeptide integrin ligands containing spirocyclic scaffolds was described including SJ749 and SJ755, the first highly selective molecules for α5β1 integrin [112,113]. Their biological effects have been characterized : they behave as potential enhancers of antibiotic efficacy by interfering with α5β1 integrin/fibronectin/M1 protein-dependent bacterial entry in epithelial cells [114], as blockers of angiogenesis [115] and as inhibitors of proliferation and migration of glioma cells [116,117]. Docking of SJ749 into a built 3D model of the binding domain of α5β1 integrin has permitted the identification of two potentially important and unique regions of this integrin compared to others [118]. This model was largely used for the rational design of highly active and selective ligands for α5β1 and αvβ3 integrins achieving a selectivity ratio as high as 10000 [119–121]. The differentiation between the α5β1 and αvβ3 integrin-dependent glioma migration modes was possible using such ligands and revealed that αvβ3 integrin antagonists increased single cell migration whereas α5β1 integrin antagonists decreased it [122]. Additionally, we showed that, unlike

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αvβ3 integrin antagonists, those selective for α5β1 integrin pushed glioma cells towards apoptosis when combined with chemotherapy or p53 activators [123–125]. Starting from a virtual combinatorial library designed to cover the chemical space specific for RGD-like compounds, Stragies et al synthesized dual α5β1/αvβ3 specific inhibitors [126]. Optimization of one lead gave JSM6427 which exhibits 10000 times more affinity for α5β1 versus αvβ3 and showed inhibitory activity in several models of pathological angiogenesis [127–129]. It was further developed for the treatment of macular degeneration but did not overpass the phase 1 clinical trial.

More recently, new β-lactam derivatives were designed to target integrins. Interestingly, the concept of integrin agonists has gradually emerged since compounds with azetidinone as the only cyclic framework increased integrin-dependent cell adhesion rather than decreasing it [130]. Substituent variations around the β-lactam core led to the discovery of selective compounds for different integrins including α5β1 and αvβ3/β5. Similar selectivity ranges were obtained for these integrins by competitive solid-phase binding assays using purified integrins and cell-adhesion based assays [131,132]. Increase in cell adhesion by integrin "agonists" was corroborated by an increase in integrin-dependent ERK signaling pathway. First characterized as racemic compounds, it was demonstrated that only (S)-enantiomers maintain the agonist activity thus revealing an important stereochemical requirement for integrin recognition and activation [133]. Whether integrin agonists could play a role as therapeutic drugs in oncology remains to be demonstrated [134].

Taking together all the data concerning these RGD-mimetic ligands, structural determinants discriminating different RGD-binding integrins have been characterized based on compound structure-activity relationships and computer-assisted docking on crystallographic integrin models. As is the case for the RGD-derived peptides, few of these original ligands are currently under investigations to check their potential anti-tumoral effects. The main knowledge concerns their ability to inhibit/increase integrin-dependent cell adhesion. Analysis of their roles on integrin signaling pathways has become an urgent need to more clearly decipher the biological cues of specific RGD-integrins in tumoral or surrounding stromal cells.

4 Current applications of RGD-ligands

Huge efforts to get more and more selective RGD-ligands led to interesting libraries of compounds. Due to the failure of the lead cilengitide to improve patient survival in clinical trials, none of them appears to be developed as anti-cancer therapeutics. Currently they are being exploited as useful tools either as tumor diagnostic or tumor targeting markers. They also serve for functionalization of biomaterials helping to solve several selective integrin-dependent cell phenotypes.

4.1. RGD-ligands for tumor diagnostic

As stated above, the input of RGD-integrins in hallmarks of cancer is largely acknowledged. Their heterogeneous expression in patient tumors closed the way to use their antagonists as therapeutics in unselected cohort of patients. Characterization of the integrin expression panel by a noninvasive way may be of importance to delineate patient subpopulations and adapt therapies. From early 2000 to 2015, huge efforts to characterize Positron Emission

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Tomography (PET) markers for αvβ3 integrin were made. Radiolabeled cyclic RGD peptides (with ^{18}F, $^{64}Cu/^{68}Ga$ tracers) were thoroughly designed and characterized (for review see [135] and [136]). Several were investigated in the clinic [137–139]. By contrast very few radiotracers specific for α5β1 integrin are available (Table 1) as this integrin moved in the forefront of cancer research more recently, in particular for its unambiguous role in neoangiogenesis. The first α5β1-selective integrin antagonist useful as a PET tracer was described in 2013 by the group of H. Kessler [140]. A selective α5β1 integrin peptidomimetic (described in [119,141]) was functionalized with the NODAGA chelator, labelled with ^{68}Ga and named FR366 [142]. FR366 has a high affinity for α5β1 integrin, a specific integrin uptake in vivo and a good tumor-to-background contrast. A further step was achieved by trimerization of an azide-functionalized pseudopeptide [143] using a 1-pot click chemistry procedure with a TRAP chelator scaffold [144]. The compound ^{68}Ga-Aquibeprin obtained by this way has affinity and selectivity for α5β1 integrins and gave high contrast PET imaging in vivo [145,146]. A similar approach was used with c(phg-isoDGR-(NMe)k peptide (see above) which was conjugated to pentynoic acid on the lysine side chain and then trimerized by the TRAP chelator. In a proof of concept experiment this compound labeled with ^{68}Ga behaved as a potential PET agent for noninvasive imaging of α5β1 expressing tumors [108]. Other PET peptidic radiotracers for α5β1 integrin were proposed (based on CRETTAVAC or KSSPHSRN(SG)5RGDSP linear peptides) but they lack an efficient accumulation in α5β1 integrin-positive tumors in vivo [147,148].

4.2. RGD-ligands for tumor targeting

Integrins appear as valuable entry door for anti-cancer drugs in a tumor selective way. Based on the overexpression of αvβ3 and α5β1 integrins in tumor vasculature and cells and their ability to get internalized through endosomes [149], strategies have been developed to achieve specific transport for therapeutics. RGD-ligands coupled to cytotoxic drugs can serve as direct carriers and are intensively investigated. The drug is coupled to the RGD peptide by a linker which may be cleaved inside the cell to allow the therapeutic effect (for review see [150,151]. Examples of RGD-ligands and cytotoxic drug complexes can be found in two recent reviews ([152,153]. Recent advances in the field included dual-functional complexes that incorporate one fluorophore on one side for imaging and a cytotoxic pro-drug on the other side [154]. But currently intense efforts are mainly directed towards nanocarriers functionalized with RGD-ligands. We will not develop here this topic as recent reviews are available to which readers can refer [155–161].

Although huge literature exists concerning the applications available with RGD-ligands for targeting tumors, they appear, to our knowledge, non-dedicated to address specifically the selectivity of RGD-integrins. The RGD-ligands mainly used in these studies have been designed to target αvβ3 integrins. From a biologist point of view, further improvements will be achieved when tailored systems will be proposed to target specifically either αvβ3 or α5β1 integrin expressing tumors.

5 Summary and outlook

In this review we focused on two RGD-integrins which are important players in oncology, the αvβ3 and α5β1 integrins. It is important to note that other RGD-integrins become under

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spotlight as for example the αvβ6 integrin [162]. Selective Inhibitors and PET tracers have been designed [163–167] which will be useful to detect and treat epithelial carcinoma for example. Integrins remain therapeutic targets in oncology (as assessed by 2 recent reviews [168,169]) but we have to reconsider their roles in the area of personalized medicine.

Our goal in this review was to give the biologist point of view regarding the chemical approaches to design selective compounds differentiating RGD-integrins in preclinical and clinical settings. According to the data (even the recent ones), the αvβ3 integrin still remains the gold standard tumoral target. Efforts are however made towards other integrins. This is an important point in order to have a better knowledge of the complex integrin world. To go further, we have to set up a virtuous circle by increasing the knowledge on one particular integrin using selective ligands and inversely to take into account this knowledge to design new ligands with improved efficacy. The biological tests are crucial as are the preclinical models. It is time to progress towards models that better reflect the clinical reality to test integrin antagonists. For example, replacing 2D by 3D sphere cultures and hence by organoids/tumoroids to test integrin antagonists is nowadays accessible steps. In addition, we have to develop high/medium throughput assays aiming to characterize the biological effects of RGD-ligands on not only integrin/cell adhesion but also on oncogenic signaling pathways. We have also to keep in mind that integrin antagonists/agonists may inhibit/activate general mechanisms which are under the control of several players. It will be of great interest to evaluate more thoroughly the benefit of their association with other targeted therapies. Transdisciplinary networks involving biologists, clinicians and chemists will greatly help to go through preclinical investigations towards clinical benefits with integrin ligands.

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Conflict of interest

The authors have no conflict of interest to declare.

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ligand	Cell line	models	references
^{68}Ga-NODAGA-peptidomimetic	Human colon carcinoma (RKO cells) Human melanoma (M21 cells)	Heterotopic xenografts in mice	Neubauer et al, 2013
^{68}Ga-NODAGA-peptidomimetic (FR366)			D'Alessandria et al, 2016
^{68}Ga-TRAP-peptidomimetic (Aquibeprin)			Notni et al, 2016
^{18}F-peptide (CRRETAWAC)	Human prostate carcinoma (DU145 cells)	Heterotopic xenografts in mice	Hauber et al, 2014
^{18}F-NOTA-peptide (KSSPHSRN(SG)$_5$RGDSP)	Murine melanoma (B16-F10 cells) Human colorectal carcinoma (SW48 cells)	Heterotopic xenografts in mice	Jin et al, 2015
^{68}Ga-TRAP-peptide (c(phg-isoDGR-(NMe)k))	Human melanoma (M21 cells)	Heterotopic xenografts in mice	Kapp et al, 2018

Table1: PET radiotracers selective for α5β1 integrin. Different xenografted tumoral cell lines have been used with differential expression of α5β1 and αvβ3 integrins. RKO cells : α5β1 +, αvβ3 -; M21 cells: α5β1 +/- and αvβ3 +; DU145 cells: α5β1 +, αvβ3 -; B16-F10: α5β1+, αvβ3 +/-; SW48 cells: α5β1 -, αvβ3 +/-.

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Legends to figures

Figure 1: **Integrins in hallmarks of cancer**. Altered expressions of integrins are detected in tumor cells but also in non tumoral cells in the tumor microenvironment. Integrin-activated signaling pathways have pro-tumoral functions.

Figure 2: RGD-integrin ligands. Integrins αvβ3 and α5β1 both recognize Fibronectin (FN). Ligands able to interfere with FN-integrin complexes have been developed mainly as integrin antagonists of cell adherence (RGD-based peptides, RGD-mimetics). Antibodies recognize epitopes in- or outside the RGD binding sites and are useful to mark integrins in inactivated or activated states. Non-RGD peptides have been developed based on the FN synergy site (PHSRN) recognized by α5β1 integrin.

Figure 3: Chemical structures of some RGD-integrin ligands. Cilengitide was the first RGD-containing cyclic peptide reaching the clinic for glioblastoma treatment [93]. LXW7 is a cyclooctapeptide described in [102]. C(phg-isoDGR-NMe)k) is described in [108]. SJ755 was one of the first small molecule specific for α5β1 integrin [112]. Compound 1 is described in [141] and compound 17 is an α5β1 integrin agonist [131].

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Figure 1

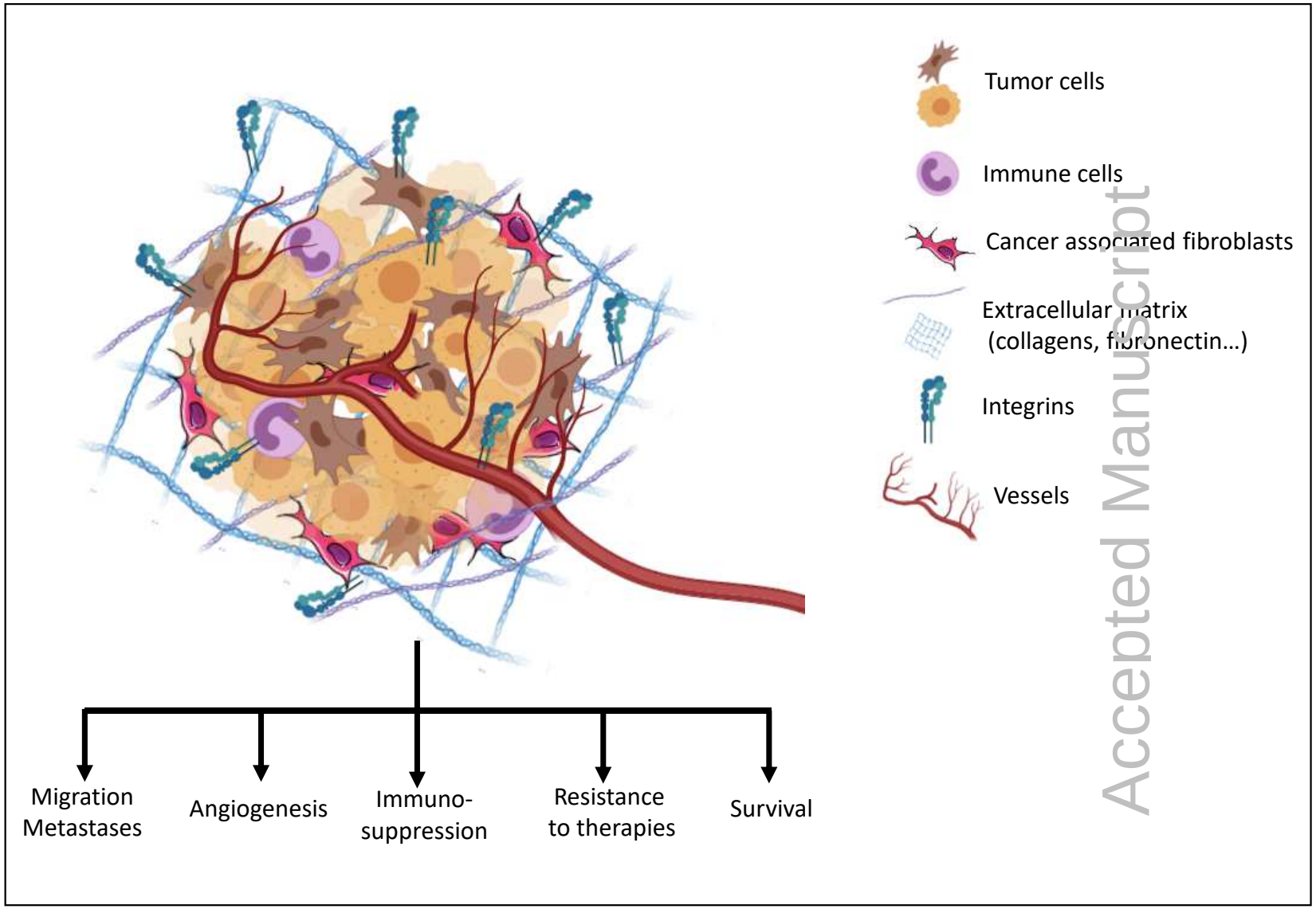

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Figure 2

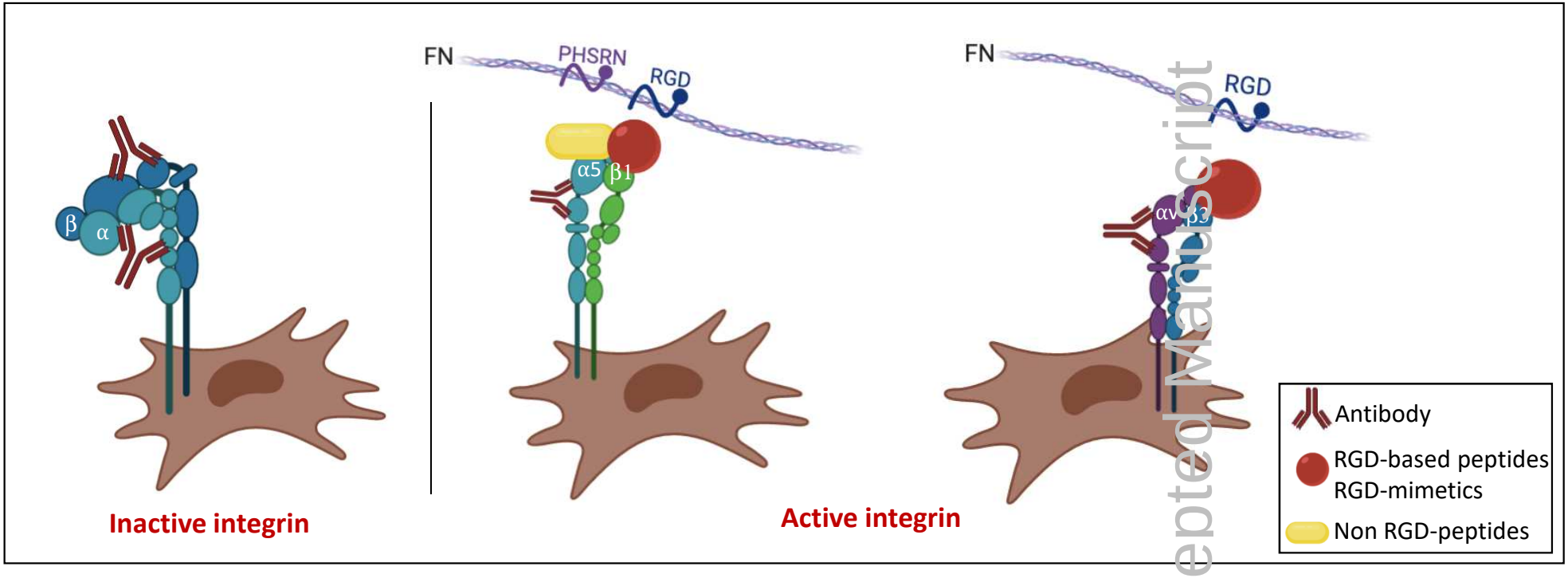

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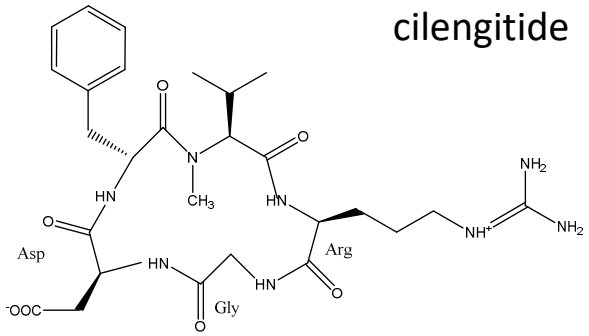
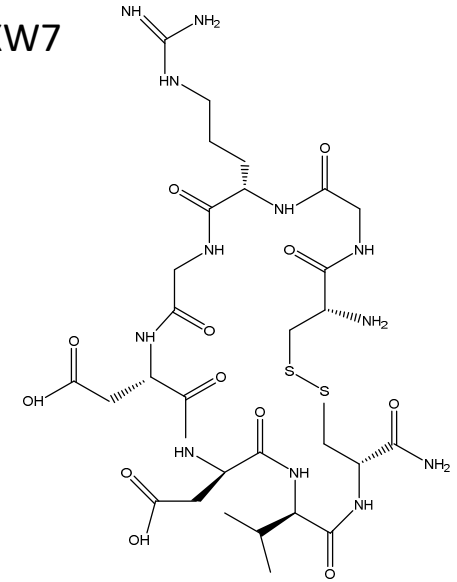
Figure 3

αvβ3 integrin

cilengitide

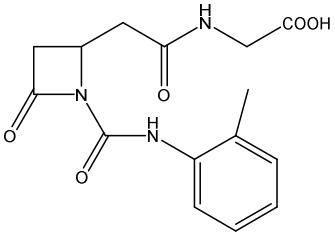


LXW7

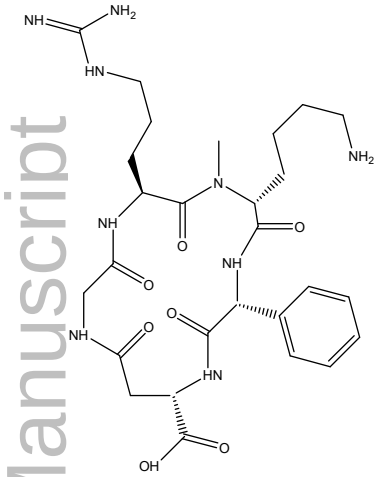


α5β1 integrin

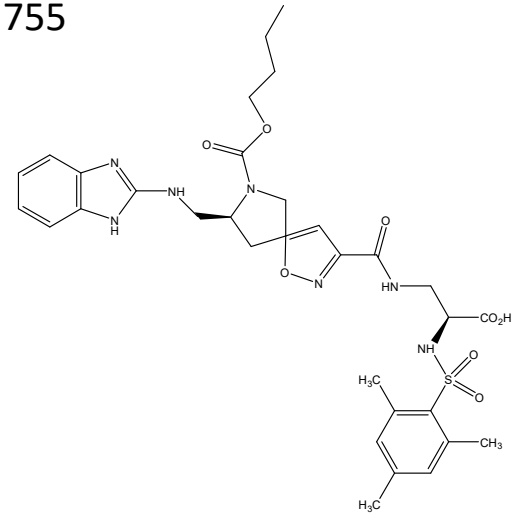
17



C(phg-isoDGR-NMe)k)



SJ755



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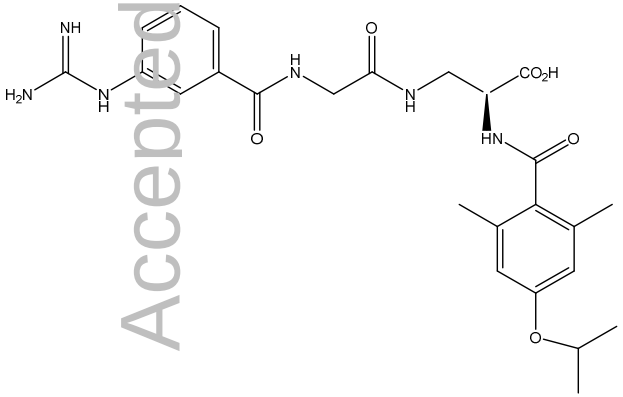

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