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RGD-Binding Integrins in Head and Neck Cancers

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Abstract: Alterations in integrin expression and function promote tumour growth, invasion, metastasis and neoangiogenesis. Head and neck cancers are highly vascular tumours with a tendency to metastasise. They express a wide range of integrin receptors. Expression of the αv and β1 subunits has been explored relatively extensively and linked to tumour progression and metastasis. Individual receptors αvβ3 and αvβ5 have proved popular targets for diagnostic and therapeutic agents but lesser studied receptors, such as αvβ6, αvβ8, and β1 subfamily members, also show promise. This review presents the current knowledge of integrin expression and function in squamous cell carcinoma of the head and neck (HNSCC), with a particular focus on the arginine-glycine-aspartate (RGD)-binding integrins, in order to highlight the potential of integrins as targets for personalised tumour-specific identification and therapy.

Keywords: integrin αv; β1; β3; β6; arginine-glycine-aspartate (RGD); metastasis; treatment resistance

1. Introduction

Head and neck cancer is a term used for any malignancies that originate from the oral cavity, oesophagus, pharynx, larynx, paranasal sinuses and nasal cavity [1]. The most recent analysis (Global Burden of Disease 2015) reported nearly 820,000 deaths from head and neck cancers occurring in 2015 [2,3]. Deaths due to lip and oral cavity cancer have increased by 32.5% over the 10 year period 2005–2015, with most other regions of the head and neck showing increases of 13–24% over the same period [3]. The most common type of head and neck cancer is squamous cell carcinoma of the mucosal surfaces (HNSCC) which accounts for about 90% of all cases [4]. Risk factors for HNSCC include the use of tobacco, alcohol and infection with human papillomavirus (HPV). Changes in incidence of oral cancer have been shown to parallel changes in tobacco use in developed and developing countries, and cases associated with HPV infection are increasing [1].

Surgery is the mainstay of HNSCC treatment, but many patients present with locally advanced disease (characterized by lymph node metastasis) which requires additional radiotherapy and/or chemotherapy [5]. Surgical cure will benefit from predictive biomarkers developed to improve identification of resectable HNSCC and lesions with malignant potential. For advanced disease, radiation treatment concurrent with chemotherapy has been recognized to improve survival [6–9], but causes severe, long-term side effects [10,11]. There is a need for both HNSCC biomarkers of disease progression and molecular targeted therapies in order to improve outcome of both surgery and overall survival from advanced metastatic disease.

Integrins are heterodimeric transmembrane glycoproteins consisting of an α-subunit and a β subunit [12]. In vertebrates, eighteen different α subunits and eight different β subunits combine to

create 24 different heterodimers [13,14]. Different types of integrins are categorized according to which cell surface, extracellular matrix (ECM) component or inflammatory ligand they bind [15]. Vertebrates have four receptor subgroups: laminin receptors (α3β1, α7β1, α6β1 and α6β4), leukocyte-specific integrins (the β2 subfamily plus α4β1, α4β7 and αEβ7), collagen receptors (α1β1, α2β1, α10β1, α11β1) and arginine-glycine-aspartate (RGD) receptors (αvβ1, αvβ3, αvβ5, αvβ6, αvβ8, α5β1, α8β1 and αIIbβ3) which recognise the triplet sequence arginine-glycine-aspartate (RGD) motif found in many ECM proteins such as fibronectin, collagen, vitronectin, osteopontin and thrombospondin [16]. Members of the RGD-binding subfamily are highly significant in angiogenesis [17] and thrombosis, and have been considered some of the most important integrin targets for drug discovery. Anti-integrin drugs which are designed to block the integrin–extracellular matrix interaction have been developed to combat a range of diseases [12,18–21]. Some of these integrin-targeted drugs, namely abciximab, eptifibatide and tirofiban targeting αIIbβ3 and natalizumab and vedolizumab targeting the α4 subfamily, are on the market. The remainder are still in clinical trials (recently summarized by Prager et al. [22]).

A number of the RGD-recognising integrins, αvβ3, αvβ5, αvβ8 and α5β1 are involved in controlling angiogenesis [23–25]. αvβ3, αvβ5 and α5β1 are expressed on activated endothelial cells during normal tissue regeneration and can become aberrantly expressed in cancers [17]. αvβ6 and αvβ8 are normally expressed on epithelial cells, notably in the lungs and, for αvβ8, the brain [26], where it is expressed on vasculature, synapses, glial cells, and dendritic spines [27]. The RGD-binding integrins support angiogenesis through survival signalling controlling cell proliferation [17], and the localisation and activation of transforming growth factor-β (TGF-β) [26–28]. In cancers, increased or altered expression of integrins on tumour cells and associated vasculature leads to tumour progression through a wide range of mechanisms, including supporting cell proliferation and tumour angiogenesis as previously mentioned, supporting the epithelial mesenchymal transition [29,30], promoting migration and invasion [31–33], interaction with the extracellular microenvironment during the metastatic process [34–36], and TGF-β activation facilitating tumour immunosuppression [26,30].

The biological potential and possible approaches to integrin targeting in general have been reviewed by many researchers [18,37–41], however, little attention has been focused on HNSCC. The role of integrins in HNSCC was last reviewed in 2005 [42]; the present review will summarise recent developments in understanding the expression and function of RGD-binding integrins in head and neck cancer progression and metastasis.

2. Integrins in HNSCC

Investigation of changes in integrin expression on tumour tissues identifies possible biomarkers for disease progression, and targets for imaging and drug delivery agents. Additionally, linking integrin expression or signalling to tumour growth, dissemination, or response to therapy identifies areas where targeted integrin inhibitors may improve prognosis The expression of RGD-binding integrins in clinical tissue samples is summarized in Table 1. Studies on these receptors will be discussed further in the following sections.

Table 1. The expression of arginine-glycine-aspartate (RGD)-binding integrins in squamous cell carcinoma tissues from the head and neck.

Integrin	SE	SCC	E	S	Ref
αvβ3	−/+	−/+	++	+	[43]
αvβ3		−			[44]
αvβ3	+	−/+			[45]
αvβ3	−/+			+++	[46]
αvβ5	+	++	++	+++	[43]
αvβ5	+	+++			[45]
αvβ5	+	++		++	[44]

Table 1. *Cont.*

Integrin	SE	SCC	E	S	Ref
αvβ1	+++	+++			[45]
αvβ6	−	++	n/a		[44]
αvβ6	++	+++			[45]
αvβ8	+++	+++			[45]
αIIbβ3					
α5β1	+	++	+	++	[43]
α5β1	−	+++			[47]
α5β1	−/+	++			[48]

Key: SE: squamous epithelium; SCC: squamous cell carcinoma; E: endothelial cells; S: stroma; − negative; −/+ weak; + moderate; ++ strong; +++ very strong; blank not tested.

2.1. The αv Integrin Subfamily

The αv subunit is present in a number of integrins involved in neoangiogenesis, migration and invasion. Associated with these processes is activation of matrix metalloproteinases (MMPs), and activation of TGF-β. αvβ3 and αvβ5 have been the focus of significant research as anti-angiogenic targets [25,49,50], but αvβ6, αvβ1, and αvβ8 are less well-investigated.

The αv integrin subunit is overexpressed in oesophageal, orbital, eyelid and periorbital squamous cell carcinomas (SCCs), and its expression is associated with tumour invasion and metastasis [51]. High expression of αv integrins promotes proliferation and invasion of oral squamous cell carcinoma (SCC) by activating the mitogen-activated protein kinase/extracellular signal-regulated kinase (MEK/ERK) signalling pathway [52]. The expression of αv integrin subunits is correlated with lymphatic metastasis of laryngeal and hypopharyngeal carcinoma, although the associated integrin β subunit that plays the major role in SCC aggressiveness was not identified [53]. In nasopharyngeal carcinoma, expression levels of integrin αv mRNA and protein are also correlated with tumour size and lymph node spread, and were significantly higher in nasopharyngeal carcinoma tissues than in non-neoplastic inflammation tissues from the same region [54].

αv expression has been shown to be higher in tissues from radioresistant nasopharyngeal carcinomas than in tissues from patients who responded to radiotherapy. In vitro, αv expression in the human nasopharyngeal cell line CNE-2 is upregulated in multi-cellular spheroids compared to standard culture conditions, and blocking αv integrin signaling in the SAPK/JNK pathway increased the radiosensitivity of multi-cellular spheroids in vitro and in vivo [55]. Downregulation of the αv integrin subunit by an antisense oligonucleotide has also been shown to inhibit the proliferation of laryngeal carcinoma cells and enhance their apoptosis [56].

Overall, αv expression appears useful as a biomarker of HNSCC tumour tissue and is amenable for biological targeting as an anticancer strategy. The development of small molecule drugs against αv subfamily integrins is feasible, but targeting a particular subfamily member has so far been a more attractive strategy to the pharmaceutical industry [40]. This requires the biological functions of individual αv subfamily members to be understood.

2.1.1. αvβ3 and αvβ5 Integrins

αvβ3 and αvβ5 are the most thoroughly studied of the αv subfamily. αvβ5 integrin expression has been found to be significantly higher in oral tumour cells and tumour stroma than in control samples of normal oral cavity squamous epithelium [43], and significantly higher in larynx and tongue SCC cells than in epithelium cells in normal tissues. Its expression is associated with lymphatic metastasis and angiogenesis [45,57]. αvβ3 was extensively expressed in endothelia with statistically significant differences between tumour and normal tissue [43]. Transfection of tongue squamous carcinoma cells (Cal27) with β3 cDNA increased expression of integrin αvβ5 alongside inducing expression of αvβ3. This increased αvβ3/β5 expression increased cell adhesion to both fibronectin

and vitronectin, migration and invasion, and induced resistance to cytotoxic drugs through reducing Src signaling [58]. Knockdown of integrin-linked kinase (ILK) or αvβ3 enhanced drug sensitivity; targeting αvβ5 did not reverse resistance [58].

In contrast, Jones et al. demonstrated that αvβ5 integrin is expressed in both normal and tumour cells of oral squamous cell carcinoma and the expression of αvβ5 was weak or absent in poorly differentiated lesions [44]. In vitro studies have implicated αvβ5 integrin in cancer regression. αvβ5 integrin-deficient cell lines lost the ability for terminal differentiation and grew in an anchorage-independent manner. When αvβ5 integrin expression was restored, the in vitro malignant phenotype was reversed [59]. Transduction of an αv-negative human SCC line (H357) with retroviral vectors encoding αv integrins showed that αvβ5-expressing cells showed enhanced apoptosis (anoikis) and suppression of AKT activity, whereas αv-negative cells and cells expressing αvβ6 did not [60]. Taken together, results from these groups suggest that αvβ5 may not be a suitable target for anti-integrin therapy due to both its role in normal skin biology, and its failure to improve efficacy or reduce resistance when targeted alongside commonly used chemotherapy agents.

Integrin αvβ3 is strongly implicated in tumour angiogenesis [61]. αvβ3 integrin overexpression has been reported on tumour-associated vessels of human carcinomas [62], in angiogenic vasculature of HNSCC mouse xenograft (including patient-derived xenografts) models [63] and in activated endothelial cells [64]. Li et al. found that chemokine receptor 7 (CCR7) and its ligand CCL19 enhance head and neck cancer cell migration and adhesion by activating αvβ3 integrin [46]. Targeting αv and β3 integrins with antibodies partially decreased the effect of the αvβ3 ligand soluble periostin on the proliferation, migration and invasion of HNSCC cells [65].

Multiple preclinical studies have shown that dual αvβ3 and αvβ5 antagonists inhibit tumour angiogenesis and tumour growth [66]. The prototypical antagonist cilengitide is a RGD-derived cyclic peptide that selectively inhibits αvβ3 and αvβ5 integrins. It demonstrated preclinical anti-tumour activity in many malignant tumours such as glioblastoma, prostate [67] and melanoma [68], progressing to clinical trials for a number of advanced cancers. Despite early promise in glioblastoma [69], cilengitide failed to improve overall survival in the phase III CENTRIC study [70] and therefore development ceased in 2015.

In a single patient case report, cilengitide with gemcitabine inhibited the growth of a highly vascularized head and neck tumour and prolonged life for more than one year, encouraging further investigation in HNSCC [71] despite the fact no preclinical studies had been reported in this tumour type. Cilengitide entered the clinic in a phase I/II trial in HNSCC [72,73]. Doses up to 2000 mg/kg were investigated in the phase I part of the trial. The combination with cisplatin, 5-fluorouracil, and cetuximab was well tolerated in patients with recurrent and/or metastatic disease. No dose-limiting or unexpected toxicities were observed and only minor adverse effects were reported [72]. The phase II part compared once or twice weekly administration of 2000 mg cilengitide in combination with cisplatin, 5-fluorouracil, and cetuximab to cisplatin, 5-fluorouracil, and cetuximab alone. Addition of cilengitide did not improve the progression-free survival in this patient population [73], and it was not further investigated.

Subsequently Heiduschka et al. reported that cilengitide in combination with cisplatin increased apoptosis and gave synergistic growth inhibition in three HNSCC cell lines (SCC25, CAL27 and FaDu). Cilengitide in combination with irradiation gave significant inhibition of colony formation [74]. In ex vivo experiments, cilengitide alone had insignificant effect on colony formation from biopsies of HNSCC [75], although the same group later found it did significantly reduce colony formation when cells were seeded on laminin [76]. Combination of cilengitide with cetuximab is effective at reducing colony formation and cytokine release from HNSCC cells, but the two targeted therapeutics did not act synergistically [76]. These results parallel those seen in other tumours i.e., cilengitide appeared promising preclinically but failed to deliver in the clinic. The difference in results observed depending on the culture environment [75,76] suggests care should be taken in the choice of preclinical models used to evaluate new integrin-targeted agents.

The trial investigators suggested that the failure of cilengitide in head and neck cancer is because HNSCC cells, in contrast to tumour endothelium, tend to express low levels of αvβ3, the integrin most tightly bound by cilengitide [43]. αvβ5 inhibition has possible pro-tumourigenic properties in both HNSCC [44,59,60] and glioblastoma [77], thus a combination αvβ3/αvβ5 targeting agent may be self-defeating in these cancers. Tumour expression of related integrins not effectively inhibited by cilengitide may also provide a mechanism for drug resistance and therapeutic failure. Moreover, Becker et al. showed that cilengitide was not distributed from the plasma to other compartments, and was mainly excreted unchanged by the kidney. Its short half-life means tumours are not exposed to therapeutic concentrations for much of the time which may promote tumour growth [78]. It has been proposed that cilengitide's poor pharmacokinetics has ultimately led to its development being discontinued [70,78] although the observation that the pan-αv antagonist GLPG0187 was not effective as a monotherapy when delivered by continuous infusion suggests other factors, such as combination of integrins targeted, may be significant [79]. The clinical failure of cilengitide makes dual inhibition of αvβ3 and αvβ5 as an antitumour strategy now difficult to justify, although αvβ3-specific agents may be worth investigating. However, inhibitors targeting both the αv subunit, and αvβ3 have been shown to increase the effectiveness of radiotherapy which may be a valuable strategy in HNSCC given the importance of radiotherapy in its treatment.

2.1.2. αvβ6

Multiple studies have found αvβ6 is overexpressed in HNSCC. One study reported overall that 95% of HNSCCs express this integrin on tumour cells, whereas αvβ3 and αvβ5 are found on tumour-associated vasculature and stromal cells, respectively [80]. αvβ6 expression is tumour specific; normal oral mucosal cells do not express the receptor [44,80,81]. Eriksen et al. demonstrated that β1, β4 and β6 integrins were upregulated in carcinomas compared to the adjacent mucosa within formalin-fixed paraffin-embedded pre-irradiation biopsies from 85 patients with head and neck squamous cell carcinomas (HNSCC) [82]. αvβ6 has also been shown to be more highly expressed in primary laryngeal SCC compared to normal epithelium cells [45] and in thyroid cancer versus normal tissue or thyroid adenomas [83]. αvβ6 is a biomarker of lymph node metastasis [83], however there is no significant relationship between expression levels and clinicopathological features of laryngeal SCC [45].

In in vitro studies, αvβ6 appears to be important for cell migration. Inducing expression of the β6 subunit in the poorly invasive human oral SCC9 cell line increased cell migration on fibronectin and invasion through basement membrane by inducing MMP-3, and increased the cell growth in vitro and in vivo [84]. Additionally, it has been shown that αvβ6 protects SCCs from anoikis by activating an AKT survival signal [60].

αvβ6 is expressed at the tips of migrating epithelial sheets during oral mucosal wound healing [85]. It promotes oral SCC invasion by facilitating the epithelial to mesenchymal transition (EMT) [86], and expression of the full-length β6 subunit has been shown to be required for EMT. Cells expressing β6 acquired a fibroblast-like morphology, increased expression of the mesenchymal marker vimentin, and reduced expression of the epithelial markers keratin and E-cadherin, whereas cells expressing a truncated form of β6 lacking the C-terminus kept their epithelial morphology and did not change vimentin or E-cadherin expression [87].

Targeting αvβ6 integrin with an inhibitory peptide significantly inhibits the proliferation of oral squamous cell carcinoma cells [80]. Xue et al. demonstrated that blocking αvβ6 integrin inhibited HSC-3 cell migration and growth in a three-dimensional collagen gel, and retarded tumour growth in vivo. While blocking αvβ6 integrin inhibited cell adhesion to latency-associated peptide of TGF-β1, combination of anti-αvβ6 with anti-α5β1 antibodies was required for complete inhibition of adhesion to fibronectin [88], thereby identifying a possible new dual inhibition strategy for HNSCC treatment. Cooperative use of αvβ1, αvβ6 and α5β1 integrins to adhere and migrate on fibronectin has previously been reported by Koivisto and colleagues [89]. The development of novel selective small molecules

binding αvβ1 [90,91], and αvβ6 and/or αvβ8 [92,93] provides the tools required to further investigate the effect of their inhibition in HNSCC and the design of potential new therapeutic and imaging agents.

2.1.3. αvβ8

Expression of the β8 and β1 integrin subunits has been shown to be slightly higher in laryngeal SCC tissue than in normal epithelium [45], confirming an earlier report of the upregulation of αv and β8 [94]. Little is known on the role of αvβ8 in HNSCC. One study has shown that overexpression of αv in oral SCC cells increased β8 level which promoted cell proliferation and MEK/ERK signalling on type I collagen. Reduction of αvβ8 levels by downregulation of integrin β8 decreased focal adhesion kinase (FAK) and MEK/ERK signalling in response to type I collagen, and thus proliferation and invasion [52]. Further study is required to establish αvβ8 as a biomarker or drug target in HNSCC.

2.2. The β1 Integrin Subfamily

Many studies have been carried out investigating the association of the β1 integrin subunit with HNSCC. The interpretation of these studies is complicated by the fact that β1 is widely expressed, and partners 12 α subunits to form members of all the integrin subfamilies: receptors for laminins, collagens, and RGD-containing ECM proteins. The β1 subunit has been proposed as a target in several of the studies described below. Development of small molecule drugs would be challenging because of the diverse range of receptors containing β1, and the potential normal tissue toxicity requires further investigation. β1 expression per se is more likely to be important as a biomarker rather than therapeutic target, which would require identification of the specific β1 integrins promoting pathology.

β1 expression has been associated with HNSCC metastasis in multiple models. β1 integrin subunit levels were significantly higher in primary tumours which had metastasised than in non-metastatic tumours and its expression was inversely correlated with overall survival. Reduction in β1 integrin levels reduced lymph node and lung metastasis in vivo and matrigel invasion in vitro by preventing activation of MMP-2 [95]. In oral and oropharyngeal SCC, β1 has been observed in 90% of tumour samples [96]. Expression of β1 enhanced oral SCC cell motility by activating the ERK pathway, and its knockdown reduced the migration activity of OEC-M1 cells and ERK phosphorylation [97].

β1 [98], α6 [99], and α7 [100] have been identified as HNSCC stem cell markers, with β1 [98] being associated with poor response to radiotherapy and increased risk of relapse and metastasis [98] (α7 has similar associations with poor prognosis and metastasis in oesophageal SCC). Moreover, targeting β1 integrin with an inhibitory antibody increased sensitivity to ionizing radiation and delayed the growth of HNSCC cell lines in 3D cultures and in xenografted mice [101], and has been shown to act by impairing repair of radiation-induced DNA double-strand breaks [102,103]. Combining the anti-β1 monoclonal antibody AIIB2 with a poly ADP ribose polymerase (PARP) inhibitor [102] or EGFR inhibitor [103] enhanced cytotoxicity and radiosensitization, providing better in vivo tumour control compared with monotherapy and irradiation in HNSCC models [103]. The combination of β1 and EGFR inhibition is proposed to act by preventing activation of MEK/ERK signalling, bypassing the effect of a single inhibitor [103]. In future, it is likely that combination therapies involving antagonists of a wider range of integrins will be developed to address drug resistance to targeted tyrosine kinase receptor antagonists, although integrin receptor inhibition as a means to promote radiosensitisation effects are more widely applicable in HNSCC treatment.

More details of the β1 integrin-interacting α integrin subunits in HNSCC became available recently. α11 is a biomarker of cancer associated fibroblasts and is overexpressed in HNSCC stroma [104]. α2, α3, α5 and α6 subunits have been shown to be overexpressed experimentally in three-dimensional extracellular matrix grown HNSCC cells [105], and clinically in tumour tissues versus normal tissues using the Oncomine database [105], and in studies of primary oral squamous cell carcinoma [48]. Expression was particularly extensive in invasive or metastatic cases, with expression being significantly associated with tumour invasion and nodal involvement [48]. Inhibition of α3 had the most significant effect in decreasing clonogenic cell survival, promoting apoptosis and

enhancing radiosensitivity, while blocking the β1 and α3 subunits resulted in increased cytotoxicity and radiosensitization compared to α3 integrin blocking alone [105]. Related integrin subunits were proposed to be able to compensate for targeting a single subunit [105], strengthening the argument for tumour-specific combination anti-integrin therapies [40].

The remaining α subunit which interacts with β1 to form a RGD-binding integrin is α8. Little is known regarding its role in any cancer. One study has found α8 to be downregulated in laryngeal SCC compared to normal tissue [94].

α5β1

Expression of α5 in oesophageal SCC patient samples is significantly correlated with lymph node metastasis, tumour size and poor overall survival of oesophageal SCC patients, and expression increased in cancer cell lines compared to normal oesophageal epithelial lines [106]. Oral SCC has also been shown to express higher levels of α5β1 integrin than normal cells [43,47], and overexpression of α5β1 was reported in many HNSCC cell lines grown in physiological three-dimensional extracellular matrix [105]. α5β1 and α2β1 were shown to be upregulated in caveolin-1 depleted cells, which are associated clinically with distant metastasis [107].

Knockdown of α5 inhibits oesophageal SCC cells growth, migration, and invasion [106]. A small molecule α5β1 antagonist effectively prevented cell migration and invasion, and reduced clonogenic survival [107], suggesting α5β1 as a target to prevent both local progression and distant metastasis in HNSCC. A Phase I trial of the peptide α5β1 antagonist ATN-161 in patients with progressive heavily pretreated solid tumours resulted in stable disease in 6/26 patients [108]. Based on these results, a Phase II clinical trial of ATN-161 in HNSCC was proposed [109] although no further progress has been reported.

2.3. αIIbβ3

αIIbβ3 is the major integrin found on platelets and has been reported to be ectopically expressed in some human tumours and cancer cell lines [110,111]. It has not been observed in HNSCC clinical samples, but has been shown to be expressed in the HSC-3 cell line, where it is involved in transmigration [111], and to mediate the metastasis of nasopharyngeal carcinoma cells through supporting tumour–platelet interactions [112]. Analysis of head and neck cancer molecular alterations and signalling pathways in patient data from The Cancer Genome Atlas identified the αIIb (and α5) integrin subunit genes to be mutated in patients with progressive disease, and the RGD-containing ligands fibrinogen, and vitronectin to be differentially expressed [113]. Further investigation of these pathways may reveal new therapeutic strategies against head and neck cancer progression, particularly since mutations in integrin genes are not commonly reported in cancers.

3. Applications of Integrin-Targeted Agents

3.1. Imaging

Targeting RGD-binding integrins for tumour imaging has been extensively studied. The majority of probes target αvβ3 (reviewed in [114,115]), although αvβ6 has also been targeted [116–118]. The challenges and opportunities posed by the non-selective nature of many RGD-binding peptides and heterogeneity of tumour expression of integrins also been considered [119].

Clinical trials of ^{18}F-galacto-RGD as a PET imaging agent to detect tumours have included HNSCC [64,120,121]. Tumour uptake was heterogenous but usually higher than background, allowing detection of most (10/12) primary tumours and some (2/6) lymph node metastases [64], and ^{18}F-galacto-RGD was proposed as an imaging agent for use in selection and monitoring of patients treated with antiangiogenic therapies. ^{111}In-RGD$_2$ has been investigated preclinically and showed promising results in selectively imaging tumour angiogenesis [63].

Non-radioactive imaging modalities based on RGD are also in development. RGD-bound near-IR emitting quantum dots allowed non-invasive imaging of xenografts up to 12 hours after injection, with the best image intensity obtained up to 6 hours after treatment [122]. Other near-IR reagents are being developed to assist with intraoperative tumour detection. Atallah et al. used AngioStamp^{TM} 800, a labelled RGD tetramer, to facilitate removal of residual tumour tissue in an orthotopic HNSCC mouse model. NIR-guided surgery increased the recurrence free survival rate by 50%. This orthotopic model allows long term follow up of mice, but the CAL33 tumour cell line used was highly αvβ3-expressing which does not represent HNSCC clinical integrin expression profiles [123]. The same group has used intraoperative αvβ3 imaging to improve the resection of lymph node metastases [124]. An indocyanine green-labelled αvβ6 antibody has also been proposed to improve resection margins by detecting tumour-specific expression at the invading tumour edge [118].

3.2. Targeted Therapeutic Delivery

The overexpression of integrins on tumour cells or associated vasculature can be used to selectively deliver a cytotoxic agent to the tumour, thereby reducing toxicity to normal tissue. The general principles and breadth of integrin-targeted drug delivery have been reviewed extensively elsewhere [125–127]. Most commonly peptides containing RGD or similar sequences are used to target integrins. The RGD-based approach is common with imaging; other sequences which require activation before binding such as NGR/iso-DGR [128], or CRGDKGPDC, which promotes tumour uptake through the CendR pathway, have also been used [129].

Specific to targeting HNSCC, Tsien et al. [130,131] have developed a peptide simultaneously targeting RGD-binding integrins using cRGDfC and a MMP-2 activatable peptide, building on their observation that the MMPs are also overexpresssd in HNSCC [132] and interact with αvβ3. cRGDfK and a small molecule αvβ3 antagonist have also been used to deliver gold nanoshells to αvβ3-expressing SCC-4 HNSCC xenografts [133]. Radiolabelled nanoshells were effective imaging agents, but could also be used to deliver therapeutic radioisotopes. The nanoshells were also used for photothermal therapy, resulting in an increase in efficacy over untargeted therapy.

αvβ3 is the most popular target for drug delivery, but it is not the only receptor targeted. Agents have been targeted to β1 using peptide $(C_{16})_2$-Glu-C_2-KSSPHSRNSGSGSGSGSGRGDSP designed to contain both the RGD and PHSRN sequences from fibronectin required for high α5β1 affinity to deliver iron oxide particles containing a photodynamic therapy agent [134,135]. Increased uptake was observed in M4e HNSCC xenografts compared to untargeted nanoparticle, suggesting the approach could be used to increase efficacy and decrease toxicity. Antibodies targeting the non-RGD binding integrin subunit β4 have also been used to deliver iron oxide nanoparticles and chemotherapy drugs to HNSCC tumours [136].

3.3. Antitumour and Anti-Metastatic Agents

Since expression of the αv and β1 subunits have been associated with proliferation [53] and metastasis [51,53,54,95,107], αvβ3 [65] and αvβ6 [80,84] associated with proliferation and invasion, targeting one or more of these receptors appears to be an attractive strategy to reduce either tumour growth or dissemination. With the exception of cilengitide, very limited preclinical studies have taken place so far [80,88,107]. The observations that inhibiting αv [55], αvβ3 [74], or β1 [101–103] increases sensitivity to irradiation is particularly significant for HNSCC as it suggests integrin-targeted therapy could be used as part of a chemoradiotherapy strategy for high risk disease.

Despite high levels of αvβ6 expression on HNSCC, preclinical and clinical studies of αvβ6 targeted agents have not yet been reported. Inhibitory antibodies and peptides have been developed and are the subject of particular interest in pancreatic cancer [137,138]. Hopefully, success here will encourage their investigation in other cancers. The interaction of αvβ6 targeting with radiotherapy also requires investigation.

Further work will be required to establish other members of the RGD-binding integrin subfamily as drug targets in HNSCC. αvβ1 and αvβ8 are relative unknowns in the anticancer field. αIIbβ3 expression on tumour tissue is controversial. Its expression on platelets is known to be significant in haematogenous metastasis [139–143], however the main route of HNSCC metastasis is through the lymph system, so αIIbβ3 targeting is unlikely to be significant in HNSCC.

4. Conclusions

RGD-binding integrins are involved in multiple biological processes that are crucial to both maintenance of body homeostasis and pathological conditions. These include cell proliferation, differentiation, apoptosis adhesion, and migration, all processes involved in cell invasion as well as angiogenesis. In terms of head and neck cancer treatment, the main problems associated with radiotherapy and chemotherapy are the profound toxicities and tumour recurrence after treatment. It is therefore important to identify targets that are highly expressed in tumour tissue but not expressed (or only at low levels) in normal tissue. The evidence to date clearly shows that integrin inhibitors can be considered molecular targeted anticancer therapy. αvβ3 appears most suitable for development of imaging agents to improve identification of margins for surgical resection of tumours. Overexpressed integrins will also be useful for targeted delivery of therapeutic agents. To date, αvβ3 and α5β1 have been targeted but the characteristic αvβ6 overexpression seen in HNSCC tumour cells [80] should also provide an important, highly specific approach.

Although published data on αvβ5 is conflicting (see [58–60] and [74]), evidence for αvβ6, αvβ8 and α5β1 indicates their inhibition should reduce tumour growth and metastasis. Investigation of other integrins in the β1 subfamily are still at an early stage; αvβ1, α8β1 (RGD binding) and α7β1, α11β1 (non-RGD-binding) integrins have potential as biomarkers of disease location and progression. The involvement of multiple integrins in the progression of head and neck cancer, and in resistance to radio- and chemotherapies, strongly indicates that improved outcomes for HNSCC patients could result from use of integrin inhibitors as precision medicine in combination with current standard-of-care treatments for high risk or progressive disease.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

AKT: protein kinase b; cRGDfC: cyclo-Arg-Gly-Asp-D-Phe-Cys; ECM: extracellular matrix; EGFR: epidermal growth factor receptor; EMT: epithelial to mesenchymal transition; FAK: focal adhesion kinase; HNSCC: head and neck squamous cell carcinoma; ILK: integrin-linked kinase; MEK/ERK: mitogen-activated protein kinase/extracellular signal-regulated kinase; MMP: matrix metalloprotease; NGR: Asn-Gly-Arg; NPC: nasopharyngeal carcinoma; PARP: poly ADP ribose polymerase; PHSRN: Pro-His-Ser-Arg-Asn; RGD: arginine-glycine-aspartate; TGF-β: transforming growth factor β; SCC: squamous cell carcinoma.

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