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Adhesion GPCRs in Tumorigenesis

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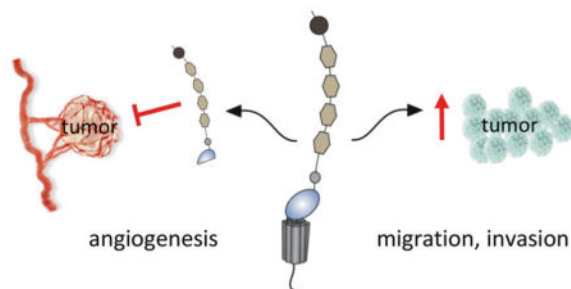
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Abstract

Alterations in the homeostasis of several adhesion GPCRs (aGPCRs) have been observed in cancer. The main cellular functions regulated by aGPCRs are cell adhesion, migration, polarity, and guidance, which are all highly relevant to tumor cell biology. Expression of aGPCRs can be induced, increased, decreased, or silenced in the tumor or in stromal cells of the tumor microenvironment, including fibroblasts and endothelial and/or immune cells. For example, ADGRE5 (CD97) and ADGRG1 (GPR56) show increased expression in many cancers, and initial functional studies suggest that both are relevant for tumor cell migration and invasion. aGPCRs can also impact the regulation of angiogenesis by releasing soluble fragments following the cleavage of their extracellular domain (ECD) at the conserved GPCR-proteolytic site (GPS) or other more distal cleavage sites as typical for the ADGRB (BAI) family. Interrogation of in silico cancer databases suggests alterations in other aGPCR members and provides the impetus for further exploration of their potential role in cancer. Integration of knowledge on the expression, regulation, and function of aGPCRs in tumorigenesis is currently spurring the first preclinical studies to examine the potential of aGPCR or the related pathways as therapeutic targets.

Graphical Abstract

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Keywords

Tumor cell migration; Tumor invasion; Metastasis; Tumor angiogenesis; Tumor therapy

1 Introduction

Cancer arises through the sequential accumulation of driver gene mutations which ultimately confer a growth advantage upon the cells in which they have occurred, leading to tumor formation through a clonal selection process. An important cancer hallmark is the ability of cancer cells to acquire invasive and metastatic properties which is associated with progression to systemic disease and reduced survival. Invasion and metastasis require tumor cell detachment and migration, extravasation into the circulation, and subsequent seeding at distant sites accompanied by altered extracellular matrix (ECM) turnover and initiation of tumor neo-angiogenesis. Each of these steps involves direct tumor cell-cell and tumor cell-microenvironment interactions, including stromal elements such as tumor-associated fibroblasts, endothelial cells, and tumor-infiltrating immune cells as well as the ECM. aGPCRs regulate cell adhesion, migration, polarity, and guidance; cellular functions that are also required for tumorigenesis. Characteristic for the aGPCRs is the large ECD with multiple adhesive folds, which is coupled to a seven-span transmembrane (7TM) domain and an intracellular domain (ICD) (see also [1–4]). Many aGPCRs are cleaved at the GPS in the juxtamembrane region which is part of and regulated by a GPCR autoproteolysis-inducing (GAIN) domain and results in an N- (NTF) and a C-terminal fragment (CTF), which remain associated at the cell surface (discussed in depth in [3, 5]). The various possible signaling scenarios resulting from this bipartite structure have been reviewed recently [6, 7] and in [8–10].

2 Software for the In Silico Interrogation of Cancer-Related Databases

A number of online resources provide user-friendly software for the rapid interrogation of large-scale cancer genomics data sets, and this in silico data mining provides useful starting information on aGPCR expression or mutation in a variety of cancer samples and cell lines.

1. The Cancer Cell Line Encyclopedia (CCLE) (www.broadinstitute.org/ccle) contains detailed genetic characterization for more than 1000 human cancer cell lines [11].

2. The cBioPortal for Cancer Genomics (www.cbioportal.org) provides analysis tools to query, analyze, and visualize large-scale cancer genomics data sets, including The Cancer Genome Atlas (TCGA) [12, 13].
3. The Human Protein Atlas (HPA) database (www.proteinatlas.org) contains high-resolution images showing the spatial distribution of proteins in dozens of normal and malignant tissues [14]. Note: the specificity of the used paraffin-suitable antibodies is not verified in depth.
4. The Catalogue of Somatic Mutations in Cancer (COSMIC) (cancer.sanger.ac.uk/cosmic) is designed to store and display somatic mutation information.
5. The SurvExpress is a biomarker validation tool and database for cancer gene expression with survival analysis [15].

3 Identification of Adhesion GPCRs in Cancer

In the last two decades, it has become apparent that changes in aGPCRs occur in cancer, either at the gene or protein expression level. Studies addressing the relevance of these changes to the tumorigenesis process are currently limited. CD97 was the first aGPCR identified to be correlated to cancer [16]. While it is not expressed in normal thyrocytes and well-differentiated papillary and in part follicular thyroid cancers, the protein was found to be elevated in anaplastic carcinomas. In the following years other aGPCRs such as GPR56 were identified to be cancer related [17]. Non-biased screens such as mRNA microarrays, genome-wide association (GWA) studies, or proteome analyses confirmed previously identified cancer-related changes in aGPCRs [18], showed alterations in new tumor entities [19], or identified new aGPCRs involved in cancer [20]. Recently, a whole-genome sequencing (WGS) approach in 441 human breast, lung, ovarian, and prostate cancers identified the aGPCRs *ADGRB3* (*BAI3*) and *ADGRL3* (*LPHN3*) among 77 significantly mutated genes [21]. Further studies are warranted to determine whether these mutations are functionally significant to cancer development or progression or simply passenger mutations.

4 Alterations in Adhesion GPCR Expression in Tumors Compared to Normal Tissue

aGPCRs may be induced, increased, decreased, or silenced in tumor compared to the corresponding normal cells. A quick way to determine whether a particular aGPCR may be present in tumor cells is to interrogate the CCLE [11]. This database shows high levels of mRNA for *ADGRA3* (GPR125), *ADGRB2* (*BAI2*), *ADGRC2* (*CELSR2*) and *ADGRC3* (*CELSR3*), *CD97*, *GPR56*, *ADGRG6* (GPR126), *ADGRL1* (*LPHN1*), and *ADGRL2* (*LPHN2*) genes in hundreds of cell lines derived from nearly all tumor entities (note: the normal corresponding cells are not included in the database). The mRNA of other aGPCRs is absent in these cell lines, including many members of the *ADGRF* and *ADGRG* families. Whether expression in cultured cell lines reflects expression in tumor cells in vivo needs to be addressed in each individual aGPCR and tumor type.

There is also evidence for changes in posttranslational modification of aGPCRs associated with cancer, which could have functional consequences. This was first shown for CD97. In normal smooth and skeletal muscle cells, the non-modified CD97 protein core is present, whereas in their malignant counterparts, i.e., in leiomyosarcoma and rhabdomyosarcoma cells, CD97 is found N-glycosylated [66, 67]. It is well known that glycosylation patterns are modified in cancer [88, 89]; therefore, it is important to determine whether such changes are relevant to the tumorigenic process for each individual aGPCR. N-glycosylation of CD97 is mapped to the adhesive EGF-like domains and is needed for the binding of the interaction partner CD55 [67], which is consistent with CD97 and CD55 often being co-expressed in cancer [53, 54, 90–93].

Regulation of aGPCR expression in stromal cells, such as tumor-associated fibroblasts and endothelial or tumor-infiltrating immune cells, as shown for ADGRA2 (GPR124) and all BAIs, may also support or suppress tumor progression. For example, BAI1 has been shown to play a role as an engulfment receptor on macrophages that can recognize apoptotic cells through binding of membrane-exposed phosphatidylserine groups to BAI1's extracellular thrombospondin repeats (TSRs) [94]. Variation in BAI1 expression on macrophages or possibly tumor cells may change the dynamics of dying tumor cell clearance from tumors, related inflammation, and cancer metabolism.

5 Soluble Adhesion GPCR and Cleavage Fragments in Tumorigenesis

As a direct consequence of the autocatalytic cleavage of aGPCRs at the GPS, as well as through (additional) independent proteolysis events within their ECD, the NTF or smaller fragments may be released from the receptor and appear in body fluids. The existence of circulating soluble NTFs is reported for GPR124 [33], BAI1 [69, 85], BAI2 [95], CD97 [96], ADGRF5 (GPR116) [97], GPR126 [98], and LPHN1 [99].

Theoretically, soluble NTFs could engage with interaction partners over large distances far away from the site at which they were released and thereby modulate heterotypic tumor-stromal interactions that may be relevant to tumorigenesis. This has been shown experimentally for the NTFs of the various isoforms of CD97, which differ in the number of EGF-like domains in their ECDs. Soluble CD97 stimulates tumor angiogenesis *in vitro* and *in vivo* through binding of integrins and chondroitin sulfate, a constituent of the ECM, in an isoform-specific manner [100]. Soluble CD97 is found at sites of inflammation [96, 101, 102], most likely released from CD97-positive immune cells, but not in body fluids of patients suffering from CD97-positive tumors [48]. These data suggest either that unknown cell-specific mechanisms control the release of the NTF or that an additional independent proteolysis event, restricted to immune cells, is necessary for this release.

In contrast to the pro-tumorigenic activity of CD97 NTFs, the NTFs of BAI1 have anti-angiogenic and anti-tumorigenic activities [69, 70] as further discussed below.

6 Biological Functions of Adhesion GPCRs in Cancer

6.1 Proliferation, Apoptosis, and Cell Cycle Regulation

Little is known about the role of aGPCRs in tumor growth, either through alteration in tumor cell division or cell death mechanisms including apoptosis. A few aGPCRs have been shown to promote cell proliferation and/or prevent apoptosis, although their role in signaling is not clarified. Downregulation of ADGRL4 (ELTD1) by microRNA-139-5p leads to an inhibition of glioblastoma cell proliferation in vitro [103]. CD97 protects fibrosarcoma HT1080 and cervical HeLa cancer cell lines from serum starvation- and staurosporine-induced intrinsic apoptosis [104].

Preliminary studies examining the effect of GPR56 on in vitro tumor cell proliferation and apoptosis have yielded contradictory results and may reflect the different cancer types and studies performed. One study reported that the knockdown of GPR56 expression induces a transient activation of apoptosis in several cancer cell lines, suggesting that GPR56 may be important for cell survival and have pro-tumorigenic functions [32]. This pro-apoptotic response could be overcome, as this research group was able to generate stable cell lines with GPR56 knockdown. These clones show a reduction in anchorage-independent growth in vitro, and some also have decreased tumor growth in vivo [32]. Similar observations were reported in the EVI-1^{high} leukemia cell line, in which GPR56 knockdown induced apoptosis although its effects on leukemia progression in vivo were not explored [22]. This pro-growth function of GPR56 contrasts with observations by another group in metastatic melanoma (A375P and MC-1) cell lines. This study suggests that GPR56 is necessary for the subcutaneous growth of these cells in mice, but does not affect their in vitro proliferation [60, 105]. The mechanisms linking GPR56 to in vivo tumor growth are found to depend on regulation of proper ECM deposition by the melanoma cells [60]. A larger panel of cell lines as well as a combination of in vitro and in vivo studies will be needed to fully understand the function of GPR56 during cancer progression.

6.2 Cell Adhesion and Interaction with ECM

Some aGPCRs have been implicated in regulating tumor cell adhesion, but potential mechanisms of these effects have not been investigated in depth. Knocking down *Gpr125* in murine myeloid sarcoma lines that harbor KRAS^{G12C} and MLL/AF10(OM-LZ) oncogenes leads to reduced myeloid sarcoma burden in mice, and a decrease in cell adhesion in culture, possibly contributing to their reduced tumorigenicity [106].

ECM proteins are large peptides that assemble into highly ordered structures modulating various aspects of cell behavior, including cell adhesion, migration, survival, and proliferation, which are key factors during tumorigenesis [107]. GPR56 NTF was discovered to bind tissue transglutaminase (TG2) [105] and collagen III [108]. It was reported that GPR56 internalizes TG2 from the surface of melanoma cells, resulting in defects in ECM deposition in melanomas [60]. This defect may contribute to the inhibitory function of GPR56 on melanoma growth and metastasis. Knocking down GPR56 inhibits the adhesion of EVI-1^{high} leukemia cells to ECM [22], and granule cells from the rostral cerebellum of *Gpr56*^{-/-} mice are defective in adhering to fibronectin and laminin [109]. The adhesion

effect of GPR56 could be blocked by its NTF: purified recombinant GPR56 NTF inhibits adhesion of glioma cells to fibronectin [82]. Taken together, the above studies point to a promoting role of GPR56 in cell-ECM adhesion, but whether this is a shared mechanism among different tumor cells and whether it contributes to the function of GPR56 in cancer progression remain to be investigated.

Several other aGPCRs such as ADGRE2 (EMR2) and GPR126 have also been shown to interact with the ECM [110–112], but the impact of these interactions on cancer has not been explored.

6.3 Cell Migration and Invasion

Among all aGPCRs, CD97 has been the most studied in the context of cell migration and invasion (see also [113] on this topic). Its association with tumor invasion is reported in several studies. It is upregulated at the invasive tumor front [42, 48, 91], and its high expression correlates with lymph node invasion [48, 54], advanced tumor stages [48, 53, 54, 91, 93], and patient survival [53, 77, 93]. The positive effects of CD97 on tumor invasion are verified in experimental models both in vitro and in vivo [49, 58]. Consistent with its function in promoting migration and invasion, CD97 is reported to enhance trans-well cell migration toward fetal calf serum in 15 different colorectal cancer cell lines [48] and toward fetal calf serum and lysophosphatidic acid (LPA) in AML cells (MV4-1 cell line) [29]. Consistently, directed cell migration and invasion stimulated by fetal calf serum are decreased following CD97 knockdown in a prostate cancer cell line (DU145) [58]. Conflicting results have been published on the effect of CD97 in a fibrosarcoma cell line (HT1080) on in vitro migration and in vivo formation of tumors and metastases: in one study CD97 overexpression increases migration in vitro and induces earlier tumor growth [49], whereas another study found the opposite [114].

Other aGPCRs have also been reported to regulate cancer cell migration. GPR56 inhibits cell migration toward stromal cell-derived factor 1 in leukemia cell lines [22], and GPR116 promotes cell migration and invasion toward serum in a breast cancer cell line (MDA-MB-231) through the Gαq-p63RhoGEF-RhoA/Rac1 pathway [65].

6.4 Angiogenesis

Several aGPCRs are found to regulate tumor angiogenesis, the process through which tumors elicit vessel formation that is critical for their sustained growth (see also [115] on this topic). The most studied one is BAI1, which inhibits angiogenesis in glioblastomas, the most malignant primary intracranial brain tumor which is incurable [69, 70, 116, 117] as well as other cancer types [36, 37, 118, 119]. The ECD of BAIs contains TSRs, and some TSRs are known to negatively regulate angiogenesis [120, 121]. Cleavage of the BAI1 ECD leads to the release of two fragments, dubbed vasculostatin-40 (Vstat40) and vasculostatin-120 (Vstat120) due to their molecular size (Fig. 1), and both were shown to suppress blood vessel formation in a variety of in vitro and in vivo assays [69, 70, 116]. When overexpressed, GPR56 can also inhibit tumor angiogenesis in melanoma, probably by blocking the secretion of vascular endothelial growth factor (VEGF) [61].

A number of aGPCRs also stimulate neovascularization in tumors. GPR124 was described first as a tumor endothelial marker (TEM5) [125]. ELTD1 is identified as part of a core angiogenic signature composed of genes whose expression jointly correlates with that of several well-recognized angiogenesis and/or endothelial cells “seed” genes, in more than 1000 human primary tumors, and is subsequently found to be essential for angiogenesis and tumor growth [34]. The NTF of CD97 promotes angiogenesis via binding to integrin $\alpha 5\beta 1$ and $\alpha v\beta 3$ and elicits a chemotactic response in endothelial cells leading to their recruitment to the tumor [100].

7 Families of Adhesion GPCR in Tumorigenesis

Table 1 gives an overview on published studies of aGPCRs in human tumors.

7.1 ADGRA

GPR124 was originally identified as an endothelial marker that is upregulated during tumor angiogenesis [126]. A soluble GPR124 fragment, probably the NTF, is shed from *GPR124*-transfected endothelial cells. Further proteolytic processing creates a protein subunit that mediates endothelial survival and subsequent tumor angiogenesis via interactions with glycosaminoglycans and the integrin $\alpha v\beta 3$ [33]. Only cell lines derived from Ewing sarcoma, chondrosarcoma, and osteosarcoma strongly express GPR124 [11]. microRNA miR-138-5p, found to be decreased in gefitinib-resistant non-small cell lung cancer (NSCLC) cell lines (PC9GR, H1975), directly targets GPR124. Thus, downregulation of GPR124 is discussed as a therapeutic approach to overcome NSCLC gefitinib resistance [127]. Knockdown of *Gpr125* reduces tumor cell aggregation and diminishes myeloid sarcoma formation induced by transplantation of immortalized acute myeloid leukemia [106].

7.2 ADGRB

The BAI1–3 proteins are predominantly expressed in the brain [116, 128]. The human *ADGRB1* (*BAI1*) gene is located on chromosome 8q24, and BAI1 is the most studied member in this aGPCR subfamily. BAI1 contains several well-defined protein modules in the N-terminus such as an integrin-binding Arg-Gly-Asp (RGD) motif followed by five TSRs, a hormone binding domain and a GAIN domain with a GPS (Fig. 1) [85]. BAI1 can be cleaved at the GPS releasing a soluble, 120 kDa anti-angiogenic NTF called vasculostatin (Vstat120), which was reported to suppress the proliferation of endothelial cells by blocking $\alpha v\beta 5$ integrin [129], reduce the migration of cultured microvascular endothelial cells in a CD36-dependent manner [70], and inhibit angiogenesis and glioma growth in vivo [69, 71, 72, 116, 130]. Subsequently, a novel more distal proteolytic processing event was identified in BAI1, and it generates a more abundant cleaved NTF containing only the RGD and the first TSR (Vstat40). This fragment was also able to inhibit angiogenesis in vitro and in vivo [71]. At the other end of the protein, the C-terminus of BAI1 contains an intracellular proline-rich region (PRR) and a terminal PDZ-binding domain (Fig. 1), which associates with a number of intracellular signaling and scaffolding proteins [131, 132], but the role of these signaling events in cancer has not been studied to date. BAI1 also plays an important

role in myogenesis, synaptic plasticity, and phagocytosis [124], but it is unclear whether any of these functions can also intersect with cancer.

BAI1 mRNA levels are consistently downregulated in primary glioma specimens and cell lines [72, 73], and in brain metastases from lung adenocarcinoma [37]. Pulmonary adenocarcinomas, gastric, and colorectal cancers also show reduced *BAI1* expression compared with normal tissue [36, 37, 44, 46]. A recent study also suggests that *BAI1* expression is significantly reduced in breast cancer and correlates with poorer patient survival [62].

The human *BAI2* and *BAI3* genes were discovered as a result of their sequence identity with *BAI1* [128] and are localized on chromosomes 1p35 and 6q12, respectively. The amino acid sequence is highly conserved in all three members; however, *BAI1* contains five TSRs, while *BAI2* or *BAI3* each contains only four. In addition, the extracellular RGD motif and the intracellular proline-rich region (PRR), a domain known to interact with Src homology 3 (SH3) and WW domain-containing proteins [133], are unique to *BAI1*. Unlike *BAI1*, the expression of *BAI2* or *BAI3* is not silenced in glioma cells [72]. Moreover, recent studies determined higher *BAI2* expression in tumor metastasis and advanced stages [134, 135].

Importantly, recent discoveries have shown that the *BAI* genes are silenced and/or undergo somatic mutations in several cancers (Fig. 1), including the lung, breast, ovarian, and brain [21, 72, 122], suggesting that eliminating their function might be required for tumor formation. Whether the identified mutations alter *BAI1* function and may contribute to tumor formation has not been investigated to date. The function of *BAI2* and *BAI3* in tumorigenesis needs to be further investigated.

7.3 ADGRC

Although the role of ADGRCs (*CELSRs*) as key components in planar cell polarity (PCP) was clarified elegantly in knockout mice, our knowledge on their expression and function in human malignancies is rather limited. Unbiased screening approaches for cancer-related proteins often scored *CELSRs* as interesting hits, but most of them await further validation as to their role in cancer. *CELSR1* is upregulated in B cells of patients with chronic lymphocytic leukemia (CLL) [25]; *CELSR1* and *CELSR3* are found to be present in gastrointestinal and brain tumors, respectively [47]; and *Celsr1* is overexpressed in Graffi murine leukemia virus (MuLV)-induced hematologic malignancies with a lymphoid subtype [24]. Gene expression profiling of mantle cell lymphoma shows that *CELSR1* is downregulated in the non-nodal form compared to other lymphomas [27], and *CELSR1* expression is increased in pure ductal breast carcinomas in situ (DCIS) compared to such tumors with invasive components [63]. Moreover, the gene locus of *CELSR1* is hypermethylated in hepatocellular carcinoma (HCC) compared to control tissue [51].

7.4 ADGRE

ADGRE1–3 (*EMR1*–3) expression is restricted to leukocytes. Thus, almost only cell lines derived from hematologic malignancies express them [11]. *EMR2* is not or rarely expressed in gastric, pancreatic, esophageal, and colorectal carcinomas [42, 136]. Here, only a subpopulation of tumor-infiltrating macrophages is strongly *EMR2* positive. Aberrant

expression of EMR2 protein is reported in breast carcinomas. It is associated with poor patient survival [64]. *EMR2* is found upregulated in glioblastoma, in particular in the mesenchymal subtype [74], and is associated with poor overall survival and an invasive phenotype [75].

EMR3 is also found to be increased in glioblastoma and associated with poor survival [76]. In colon cancer EMR3 is restricted to tumor-infiltrating immune cells [137].

CD97 is the only ADGRE family member whose expression is not restricted to immune cells. In human, its expression varies in cells of epithelial and mesenchymal origin from negative, as in keratinocytes and thyrocytes, to low in enterocytes, and high in pneumocytes and leukocytes. Cancer cell lines are almost all moderately or strongly *CD97* positive, suggesting that, compared to normal tissues, CD97 is frequently induced and/or increased in the corresponding malignancies. Low *CD97* levels are found only in cell lines derived from neuroblastomas. Small cell lung cancer (SCLC) cell lines are nearly all CD97 negative, while non-small cell lung cancer (NSCLC) cell lines are strongly CD97 positive, a fact that could be helpful in discriminant analysis on lung cancers.

In thyroid cancer, CD97 is induced and expression levels correlate with malignant progression [16, 57], i.e., only few papillary and follicular thyroid carcinomas are CD97 positive, whereas most anaplastic cancers strongly express CD97. In normal human intestinal epithelial cells, CD97 resides at low levels in lateral cell contacts, whereas in colorectal cancer, the molecule is increased. In part, this upregulation parallels its new cellular location within the cytoplasm [48, 49]. Overexpression of CD97 in scattered tumor cells is observed at the tumor invasion front [48]. The presence of these CD97-positive scattered tumor cells correlates with higher tumor stage and higher lymphatic vessel infiltration, both prognostic factors in colorectal cancer. CD97 is also upregulated in gastric cancer [42]. Further supporting an oncogenic role, in mouse models of colorectal and gastric cancer, CD97 supports local tumor growth and promoted metastatic spread [49, 138].

In gall bladder carcinoma, CD97 expression is an independent risk factor for overall survival [53]. CD97 is highly expressed in tumor cells of poorly differentiated pancreatic ductal adenocarcinoma and in tumor-infiltrating leukocytes in chronic pancreatitis samples, but not on normal pancreatic epithelial cells [139]. In a study of 37 pancreatic cancer patients, CD97 correlates with aggressiveness and was associated with prognosis [54]. In esophageal cancer CD97 is among the 19 genes with promoter hypomethylation and upregulation [140].

CD97 mediates invasion in prostate cancer cells, at least in part, by associating with lysophosphatidic acid receptor 1 (LPAR1), leading to enhanced LPA-dependent RHO and extracellular signal-regulated kinase (ERK) activation. Consistent with its role in invasion, depletion of CD97 in prostate cancer cells (PC3 cell line) results in decreased bone metastasis without affecting subcutaneous tumor growth. CD97 and LPAR1 are significantly co-expressed in clinical prostate cancer specimens.

CD97 is highly expressed in glioblastoma (WHO grade IV) and has prognostic significance in two independent cohorts of 187 and 539 glioblastoma patients, respectively [18, 77]. CD97 is also identified as a potential biomarker for glioma-initiating cells through an in vivo

phage display screen [78], suggesting it represents a potential new therapeutic target [77]. In contrast, CD97 expression is minimal in WHO grade II and III astrocytomas [79]. Experimental data suggest that CD97 regulates tumor cell invasion in glioblastomas. Decreasing CD97 by siRNA reduces migration and invasion, but not proliferation in two glioblastoma cell lines [77]. Mass spectroscopy-based proteomic analysis showed that CD97 is enriched in membrane fractions of invadopodia, actin-rich protrusions, of invasive glioblastoma cells [18]. The mechanism underlying CD97-mediated promotion of invasion of glioblastoma cells is under investigation. Suppression of Wilms' tumor gene product WT1 by siRNA led to a decrease in the invasiveness of glioblastoma cell lines paralleled by a suppression of *CD97* RNA [80].

Further evidence for a role of CD97 in tumorigenesis comes from screening studies demonstrating that it is a direct target of the tumor suppressor microRNA-126 in the breast cancer cell line MDA-MB-231 [141].

Additionally, evaluation of CD97 expression in leukemia is highly informative. Gene expression studies and characterization of the leukemia cell surface proteome identified CD97 as a marker for minimal residual disease in acute lymphoblastic leukemia (ALL) [19]. CD97 expression also accounts for the most informative differences between normal and malignant cells in ALL [26]. CD97 has further been identified as a leukemic stem cell marker in acute myeloid leukemia (AML) [28]. In AML, expression levels of CD97 are associated with internal tandem duplications within the juxtamembrane region of the FMS-like tyrosine kinase receptor FLT3 (LFT3-ITD) [29].

Whether the function of CD97 in tumorigenesis could be modulated by other interaction partners such as LPAR1 is unknown. CD55 [142], chondroitin sulfate B [110], $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins [100], and CD90 [143] bind distinct sites within the NTF of CD97 at immune cells; nothing is known whether these interaction partners also bind CD97 in tumors. A few articles have been published on co-expression of CD55 and CD97 in solid tumors but not on the resulting functional consequence [53, 54, 91–93].

7.5 ADGRF

GPR116, highly enriched in fetal and adult lung [144–146], is part of a gene expression signature that differentiates adenocarcinoma of lung and breast origin in effusions [20]. Knockdown of *GPR116* suppresses migration and invasion, whereas ectopic expression enhances invasion of the breast cancer cell line MDA-MB-231 [65]. *GPR116* promotes constitutively breast cancer metastasis via the $G\alpha_q$ -p63RhoGEF-Rho GTPase pathway [65]. Its expression is significantly correlated with breast tumor progression, recurrence, and poor prognosis.

7.6 ADGRG

Among the ADGRG members, *GPR56* is the most studied in the context of cancer progression. It was reported in 1999 to be downregulated in highly metastatic melanoma cell lines compared with poorly metastatic lines [17]. Its inverse correlation with metastatic potential was evaluated in more depth later in an experimental metastasis model which shows the downregulation of *GPR56* in several highly metastatic melanoma derivatives

compared with their poorly metastatic parental line (A375P). Re-expression of GPR56 inhibits the growth and metastasis of melanoma cells [105], supporting a tumor suppressor role for GPR56 in melanoma. Mechanistic studies in one of the metastatic derivatives (MC-1) revealed that GPR56 overexpression inhibits the activation of PKC α , resulting in the suppression of VEGF secretion from melanoma cells, leading to angiogenesis inhibition [61]. In contrast to the full-length receptor, expression of the CTF of GPR56 or deletion of the serine threonine proline (STP)-rich segment (STP-GPR56) leads to enhanced activation of PKC α , VEGF secretion, and angiogenesis. These opposing functions of GPR56 and its CTF indicate that GPR56 might exist in different activation states, although the signaling mechanisms of GPR56 in cancer have not been elucidated. The NTF of GPR56 binds to tissue transglutaminase (TG2) [105] and collagen III [108]. The interaction between GPR56 and collagen III in cancer progression is not reported. TG2 is a cross-linking enzyme in the ECM and thought to play pleiotropic roles in cancer progression [147]. The interaction of GPR56 with TG2 in melanoma growth was evaluated recently in a xenograft model using the immunodeficient *Tg2*^{-/-} mice [60]. TG2-knockdown melanomas growing in these mice are depleted of TG2 in both cancer cells and stroma and found to be much smaller than the control tumors growing in *Tg2*^{+/+} mice, arguing that TG2 promotes melanoma growth. This tumor-promoting function is abolished by GPR56 overexpression, probably via receptor-mediated TG2 internalization from the cell surface [60]. Whether GPR56 functions similarly in metastasis and in other cancer types remains to be determined.

In contrast to the situation in melanoma, GPR56 is often found upregulated in other cancer types relative to the corresponding normal tissues [32, 43, 148, 149], so perhaps its function in cancer is cell of origin and/or stage specific. Knocking down of *GPR56* induces transient apoptosis and reduces the in vitro growth of several cancer cell lines derived from colon (HCT116), melanoma (M14), and cervix (HeLa). Notwithstanding this pro-apoptotic response, the authors were able to derive stable GPR56 knockdown in all the cancer cell lines they tested, including those from the cervix (HeLa), colon (HCT116), melanoma (A2058 and M14), ovary (OVCAR3 and OVCAR8), prostate (PC3), pancreas (AsPC1), and lung (NCI-H460). A reduction in anchorage-independent growth in vitro was observed in all the knockdown lines, and decreased tumor growth in vivo was observed in the knockdown of A2058, PC3, and HCT116 cell lines [32]. This study suggests that GPR56 plays a tumor-promoting role in certain cancer cells. Similar pro-proliferation effects of GPR56 are observed in EVI1^{high} leukemia cell lines AML1 and HNT34, in which knocking down of *GPR56* enriches cells at the subG1 phase of the cell cycle and induces apoptosis [22].

The discrepancies among the above studies need to be followed up in future investigations. It is possible that GPR56 has both pro- and anti-growth functions during cancer progression, for example, via distinct binding partners. In fact, in a recent study [60], knocking down of both GPR56 and TG2 leads to a much more severe reduction in subcutaneous growth of MC-1 cells than *TG2*-knockdown alone, suggesting that GPR56 may have tumor-promoting functions in MC-1 cells in the absence of TG2. This effect of GPR56 was not observed in the WM115 melanoma cell line, pointing again to the complexity of GPR56 function in cancer.

Very recently, GPR56 was identified as a novel and stable marker for leukemic subpopulations with high repopulating capacity, a key feature attributed to leukemia stem cells, for the majority of acute myeloid leukemia (AML) samples [30]. High GPR56 expression was significantly associated with high-risk genetic subgroups and poor outcome.

The second member of the ADGRG group, *GPR64*, was found upregulated in Ewing sarcomas (ES) compared with normal tissues and other sarcomas [68]. *Gpr64* knockdown in Ewing sarcoma lines led to a reduction in tumor growth and metastasis. This tumor-promoting function of Gpr64 was mediated by the induction of placental growth factor (PGF) and matrix metalloproteinase 1 (MMP1) [68]. The mRNA of GPR64 was detected at high levels in cell lines from prostate cancer, non-small cell lung cancer (NSCLC), and melanomas and at moderate to low levels in cell lines from brain, ovary, breast, and colon cancers [68]. It was also identified as a marker for a subgroup of medulloblastomas characterized by overactive WNT signaling [87]. *GPR64* silencing in the highly motile cancer cell lines Hs578T and MDA-MB-231 resulted in a reduction of cell adhesion and migration [150].

ADGRG4 (GPR112) was identified as a marker for neuroendocrine carcinoma cells [151] and was predicted to be one of the candidate genes for targeted therapy of ileal carcinoids [50].

None of the other ADGRG members (ADGRG3, GPR97; ADGRG5, GPR114; GPR126; ADGRG7, GPR128) have been reported to affect tumorigenesis or be dysregulated in cancer samples.

7.7 ADGRL

The CCLE predicts high expression of LPHN1 and LPHN2 in many tumors [11]. A gene identification study in breast tumors led to *Latrophilin-1* [59]. Indeed, a number of breast tumor cell lines apparently overexpress the gene [152]. An invasion-associated four-gene signature including *Latrophilin-1* obtained from the NCI-60 cell line panel has significant prediction in non-small cell lung cancer (NSCLC) [40]. A genome-wide screen between a recurrent muscle-invasive cis-platin-resistant urothelial bladder carcinoma and its adjacent non-tumor tissue found cancer-associated alternative splicing with differential exon usage for *LPHN2* [153]. Linkage studies have revealed numerous *Latrophilin-3* loss-of-function mutations in breast, lung, ovarian, and prostate cancers [21].

ELTD1 is a regulator of physiological and tumor angiogenesis in vitro and in vivo [34]. ELTD1 showed higher expression on endothelium in peritumoral vessels compared to vessels of matched normal tissues [34]. Silencing of *ELTD1* in human ovarian and colorectal cancer xenografts implanted in mice inhibited tumor growth [34]. The transcriptome of the microvasculature associated with glioblastoma identified *ETLD1* as 1 of 95 upregulated genes [35]. Indeed, ELTD1 can be used as a vascular biomarker in glioblastoma [84]. It displays higher expression in high-grade compared with low-grade gliomas. miR-139-5p suppresses glioma cell proliferation by targeting *ELTD1* and regulating the cell cycle [103].

7.8 ADGRV

To identify molecular biomarkers associated with low-grade glioma-associated epileptic seizures, one of the initial symptoms of this tumor, RNA sequence data were collected [154]. A lower expression level of *ADGRV1* (*VLGR1*) was found in patients with epileptic seizures compared to seizure-free patients and was confirmed by quantitative RT-PCR.

8 Tumor Therapy and Adhesion GPCRs

Accumulating evidence of the direct involvement of aGPCRs in tumor pathogenesis provides a solid foundation for their further study as potential therapeutic targets. Some aGPCRs are silenced with tumor formation and appear to have tumor suppressor activity (BAI1), while others are overexpressed in cancer and may act as oncogenic factors (CD97, GPR116, ELTD1).

The observation that BAI1 is epigenetically silenced in malignant glioma [72, 155], along with the recent findings of multiple somatic point mutations in the BAI1-3s in several cancers [21, 122], suggests that tumorigenesis may select for BAI1 silencing or inactivation. Importantly, exogenous restoration of BAI1 expression reduces growth and vascularization of tumors derived from gliomas and pancreatic and renal cell carcinomas [36, 119, 156, 157]. Taken together, these studies suggest that BAI1 fulfills the criteria for a *bona fide* tumor suppressor, and there is significant potential for BAI1 and its extracellular fragments as therapies for the treatment of human cancers. *BAI1* is silenced in glioblastoma multiforme due to methylation of a CpG island in the gene regulatory region, which leads to binding of methyl-CpG-binding domain protein 2 (MBD2) and transition to a suppressive chromatin conformation [72]. Treatment of glioma cells with 5-aza-2'-deoxycytidine (5-Aza-dC) or knockdown of *MBD2* by shRNA resulted in reactivation of *BAI1* expression and restoration of BAI1 functional activity in that it conferred potent anti-angiogenic activity to glioma cell conditioned media in vitro and in vivo [72]. These findings have therapeutic implications since inhibiting MBD2 could offer a strategy to reactivate BAI1 expression and suppress tumor growth. Sequence-specific antisense inhibitors of MBD2 have been shown to inhibit both anchorage-independent growth of human cancer cell lines in vitro and the growth of human tumor xenografts in vivo [158, 159]. At present, epigenetic approaches in cancer therapy have focused primarily on inhibitors of the DNA methyltransferases and histone modifiers (e.g., HDACs). Thus, targeting MBD2 to reactivate BAI1 may represent a novel promising cancer therapeutic intervention.

In contrast to BAI1, some aGPCRs are overexpressed in tumors. Expression of CD97 was found in human thyroid carcinomas, but not in the corresponding normal thyrocytes [16, 57]. In human thyroid cancer cell lines, CD97 depletion reduced Rho-GTP and decreased lysophosphatidic acid (LPA)-stimulated invasion [57]. Similarly, GPR116 protein expression correlates with clinical progression stages in breast cancer and peaks in tumors with distant metastases [20, 65]. Knockdown of GPR116 in highly metastatic breast cancer cells (MDA-MB-231) suppressed cell migration, invasion, and metastasis in vivo [65]. These studies suggest that modulation of aGPCR expression in tumors may be effective therapeutics. A recent study identified ELTD1 as a novel angiogenesis regulator [34, 35, 84]. ELTD1 is upregulated in tumor endothelial cells and is a good prognostic marker. Moreover, targeting

Eltf1 blocks tumor angiogenesis and substantially inhibits tumor growth in vivo [34]. The function of GPR56 in different cancer types was found to differ, with both pro- and anti-tumorigenic properties, so the therapeutic implication of targeting GPR56 in cancer in general awaits careful evaluation. ADGRG4 (GPR112) was reported to serve as a marker for neuroendocrine carcinoma cells [151] and proposed a therapeutic target for treating ileal carcinoids [50].

In summary, alterations in the expression pattern of different aGPCRs have been documented in several cancers, suggesting that they may potentially serve as biomarkers of disease or therapeutic targets. Undoubtedly, the interest in the therapeutic targeting of the aGPCR family will continue to grow in the upcoming years, but first requires a more comprehensive characterization of their fundamental biological function.

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Abbreviations

| | |
|-------------|-------------------------------|
| CCLE | Cancer Cell Line Encyclopedia |
| CTF | C-terminal fragment |
| ECD | Extracellular domain |
| ECM | Extracellular matrix |
| GAIN | GPCR autoproteolysis inducing |
| GPS | GPCR-proteolytic site |
| NTF | N-terminal fragment |
| TG2 | Tissue transglutaminase |
| TSR | Thrombospondin repeat |

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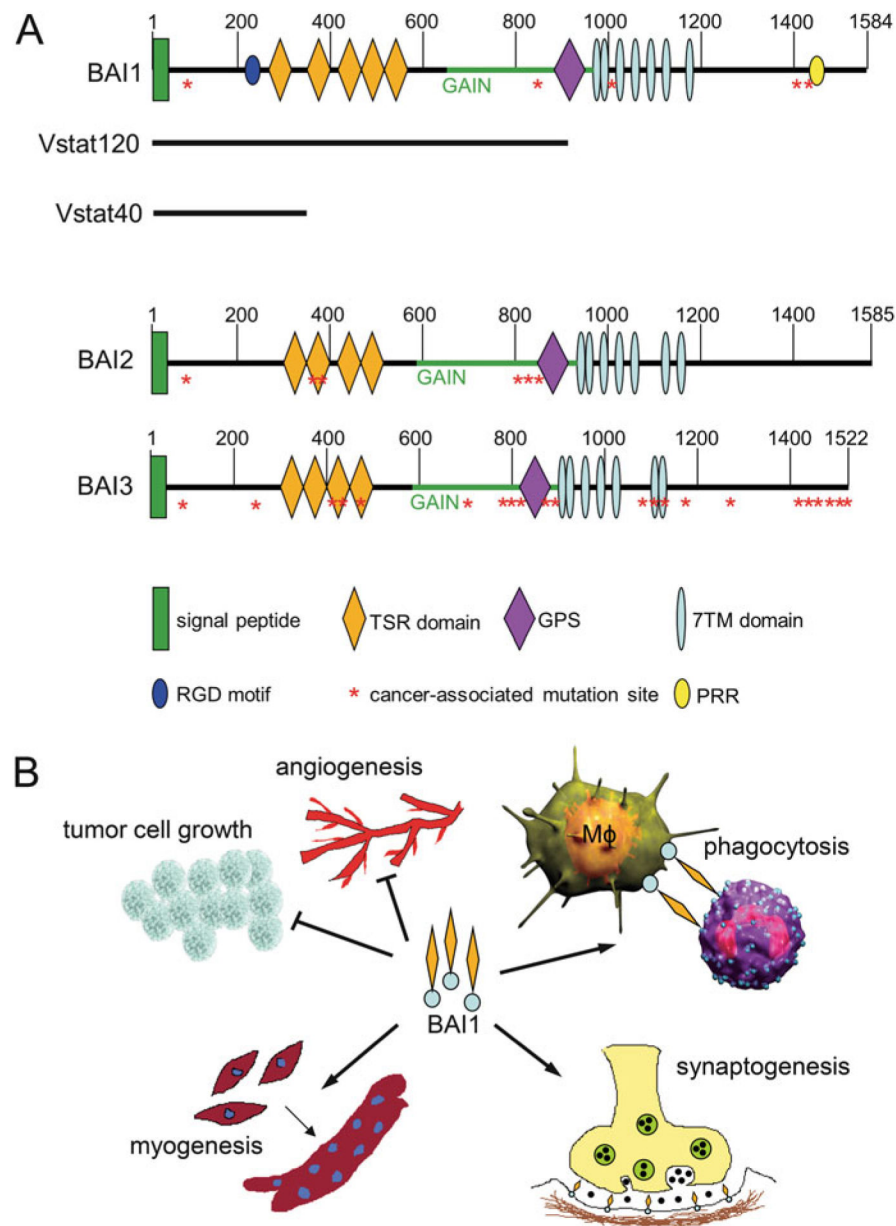


Fig. 1. Structure and functions of BAIs (ADGRBs). **(a)** Schematic of the three BAI proteins, representing the major known structural and functional features as well as known proteolysis events that generate either Vstat120 [69] or Vstat40 [71], and cancer-associated somatic mutations [21, 122]. Abbreviations: *TSR* thrombospondin type 1 repeat, *GAIN* GPCR autoproteolysis-inducing domain [123], *GPS* GPCR-proteolytic site, *7TM* seven-transmembrane region, *RGD* Arg-Gly-Asp integrin-binding motif, *PRR* proline-rich region. **(b)** Multiple functions of BAI1: BAI1 inhibits tumor cell growth in part by inhibiting angiogenesis. Moreover, its TSRs interact with exposed phosphatidylserine at the outer leaflet on apoptotic cells and elicit the engulfment in macrophages (M ϕ). BAI1 also

promotes myogenesis. Deficiency of BAI1 promotes PSD-95 degradation at the synapses and induces enhanced long-term potentiation. Figure adapted from [124]

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Table 1

Expression of adhesion GPCRs in tumors

| System | Tissue | Tumor | ADGRA2 GPR124 | ADGRB1 BAI1 | ADGRB2 BAI2 | ADGRB3 BAI3 | ADGRG1 CELSRI | ADGRG3 CELSR3 | ADGRE2 EMR2 | ADGRE3 EMR3 | ADGRE5 CD97 | ADGRF5 GPR116 | ADGRG1 GPR56 | ADGRG2 GPR64 | ADGRG4 GPR112 | ADGRL1 LPNH1 | ADGRL4 ELTD1 |
|-----------------|-------------------------|------------------------------------|------------------|----------------|----------------|----------------|------------------|------------------|----------------|----------------|----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|
| Hematopoietic | | Leukemia | | | | | | | | | | | [22, 23] | | | | |
| | | B-cell leukemia | | | | | [24] | | | | | | | | | | |
| | | Chronic lymphocytic leukemia (CLL) | | | | | [25] | | | | | | | | | | |
| | | Acute lymphatic leukemia (ALL) | | | | | | | | | [19, 26] | | | | | | |
| | | Mantle cell lymphoma (MCL) | | | | | [27] | | | | | | | | | | |
| | | Acute myeloid leukemia (AML) | | | | | | | | | [28], [29] | | | [30] | | | |
| | Other leukemias | | | | | | | | | | | [31] | | | | | |
| Cardiovascular | Heart | | | | | | | | | | | | [32] | | | | |
| | Endothelial cells | | [33] | | | | | | | | | | | | | | [34, 35] |
| Respiratory | Lung | | | [36, 37] | [21] | [38] | | | | | | | [32, 39] | | [40] | | |
| | | | | | | [41] | | | | | | | | | | | |
| Digestive | Esophagus | | | | | | | | | | | | | | | | |
| | Stomach | | | [44] | | | | | | | [42] | | [43] | | | | |
| | Large bowel | | | [46] | | | [47] | | | | [48, 49] | | [32] | | | | |
| | Neuroendocrine | | | | | | | | | | | | | | [50] | | |
| | Liver | | | | | | [51] | | | | | | [52] | | | | |
| | Gall bladder, bile duct | | | | | | | | | | | | | | | | |
| Urinary | Pancreas | | | | | | | | | | | | | | | | |
| | Kidney | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | |
| Endocrine | Thyroid | | | | | | | | | | | | | | | | |
| | Prostate | | | | | | | | | | [16, 57] | | | | | | |
| Reproductive | Ovary | | | [21] | [21] | | | | | | | | | | | | |
| | Uterus | | | | | | | | | | | | | | | | |
| | | | | | | | | | | | | | | | | | [59] |
| Skin | Skin tumors | | | | | | | | | | | | | | | | |
| | Melanoma | | | | | | | | | | | | | | | | |
| Musculoskeletal | Mammary | | | [21] | | [63] | | | | | | | | | | | |
| | Skeletal muscle | | | | | | | [64] | | | | | | | | | |

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| System | Tissue | Tumor | ADGRA2 GPR124 | ADGRB1 BAI1 | ADGRB2 BAI2 | ADGRB3 BAI3 | ADGRG1 CELSR1 | ADGRG3 CELSR3 | ADGRE2 EMR2 | ADGRE3 EMR3 | ADGRE5 CD97 | ADGRF5 GPR116 | ADGRG1 GPR56 | ADGRG2 GPR64 | ADGRG4 GPR112 | ADGRL1 LPHN1 | ADGRL4 ELTD1 | |
|----------------------|-----------------|-------------------------------|-------------------------------|----------------|----------------|----------------|------------------|------------------|----------------|----------------|-----------------|------------------|-----------------|-----------------|------------------|-----------------|-----------------|--|
| Brain/nervous system | Smooth muscle | Leiomyosarcoma | | | | | | | | [67] | | | | | | | | |
| | Bone related | Ewing sarcoma | | | | | | | | | | | [68] | | | | | |
| | Neurons, glia | Brain tumors | Brain tumors | [37] | | | | [47] | | | | | [32] | | | | | |
| | | Glioblastoma multiforme (GBM) | Glioblastoma multiforme (GBM) | [69-73] | | | | [74, 75] | [76] | | [18, 21, 77-81] | | [82, 83] | | [84] | | | |
| | | Malignant glioma | Malignant glioma | [85, 86] | [72] | [72] | | | | | | | [82] | | | | | |
| | | Astrocytoma | Astrocytoma | | | | | | | | | | [82] | | | | | |
| | Medulloblastoma | Medulloblastoma | | | | | | | | | | | [87] | | | | | |

Most studies were performed at the protein level. Only aGPCRs with published data are included. The direction of regulation is not indicated because of the partly complex investigated scenarios