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Review

The Emerging Role of Adhesion GPCRs in Cancer

Abanoub A. Gad and Nariman Balenga*

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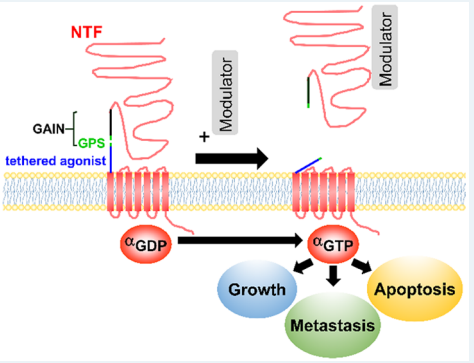
ABSTRACT: Aberrant expression, function, and mutation of G protein-coupled receptors (GPCRs) and their signaling partners, G proteins, have been well documented in many forms of cancer. These cell surface receptors and their endogenous ligands are implicated in all aspects of cancer including proliferation, angiogenesis, invasion, and metastasis. Adhesion GPCRs (aGPCRs) form the second largest family of GPCRs, most of which are orphan receptors with unknown physiological functions. This is mainly due to our limited insight into their structure, natural ligands, signaling pathways, and tissue expression profiles. Nevertheless, recent studies show that aGPCRs play important roles in cell adhesion to the extracellular matrix and cell−cell communication, processes that are dysregulated in cancer. Emerging evidence suggests that aGPCRs are implicated in migration, proliferation, and survival of tumor cells. We here review the role of aGPCRs in the five most common types of cancer (lung, breast, colorectal, prostate, and gastric) and emphasize the importance of further translational studies in this field.



KEYWORDS: *adhesion GPCRs, metastasis, cancer, extracellular matrix, proliferation*

G PROTEIN-COUPLED RECEPTORS AND THEIR ROLE IN CANCER

G protein-coupled receptors (GPCRs) are the largest superfamily of cell surface receptors in the human genome and are implicated in various biological processes including sensory perception (vision, olfaction, taste), cellular adhesion, angiogenesis, development, and hormonal regulation, among others.[1] Structurally, GPCRs are defined by seven-transmembrane domains with alternating extracellular and intracellular loops[2−4] and are grouped into five classes: rhodopsin-like, secretin, glutamate, adhesion, and frizzled (GRAFS classification).[5] The diverse stimuli of GPCR signaling and the consequent physiological events make these receptors one of the most intriguing pharmacological targets. To date, approximately 34% of drugs in the global market target 108 unique GPCRs[6] and about 56% of the nonolfactory GPCRs have yet to be studied in a clinical trial.[6] This underscores the high potential of GPCRs as novel targets in various therapeutic areas.

Compelling evidence suggests that GPCRs play major roles in cancer including growth, migration, metastasis, invasion, and survival.[7−10] GPCRs and their cognate heterotrimeric G proteins and signaling circuits are implicated in breast, lung, colorectal, prostate, and brain tumors, among many others.[9,11−13] Despite the recent advancements in our structural and functional knowledge of GPCRs,[14−16] only eight FDA-approved drugs target GPCRs for cancer therapy.[17] This underscores the need to explore the function of both deorphanized and orphan GPCRs in tumorigenesis, with a mission to reveal novel therapeutic targets. Adhesion GPCRs (aGPCRs) form the second-largest (33 members) family of GPCRs, of which only 11 members have been deorphanized.[18] The role of some members of the aGPCR family as modulators of proliferation, metastasis, and cancer cell communication is gradually being appreciated.[19]

STRUCTURE AND MECHANISMS OF ACTIVATION OF AGPCRS

aGPCRs maintain the seven-transmembrane structure but are uniquely identified by a large N-terminal fragment (NTF) (Figure 1). In some aGPCRs, NTF contains several domains including EGF-like, cadherin, pentraxin, and leucine-rich repeats, which enable cells to interact with adhesion molecules (e.g., ADGRE5 with integrins[20]) or extracellular matrices (ECM) (e.g., ADGRG1 with collagen III;[21] ADGRG6 with collagen IV[22]). aGPCRs, except ADGRA1, include a GPCR Autoproteolysis-Inducing domain (GAIN), which is located N-terminally to the first transmembrane domain.[18] Within the GAIN domain is a highly conserved GPCR proteolytic site (GPS) and a stretch of residues that connects GPS to the first transmembrane domain.[23] Proteolytic cleavage at the GPS during protein translation divides aGPCRs into NTF and C-

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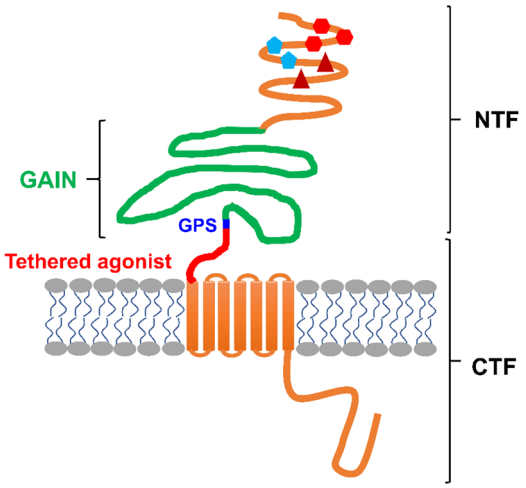

Figure 1. General structure of an aGPCR. All aGPCRs, except ADGRA1, contain a GPCR Autoproteolysis-Inducing domain (GAIN)[23] that includes the GPCR proteolysis site (GPS) and a tethered agonist sequence.[30] The cleavage at GPS results in a two-subunit molecule, including an N-terminal fragment (NTF) and a C-terminal fragment (CTF) that remain associated via noncovalent interactions. In some aGPCRs, NTF includes additional domains such as EGF-like, cadherin, pentraxin, and leucine-rich repeats. These domains interact with other cell adhesion molecules and extracellular matrices, by which they orchestrate the intracellular signaling.

terminal fragments (CTF), which remain associated via noncovalent interactions.

Several modes of activation of aGPCRs have been proposed, some of which are unique to specific aGPCRs and cellular contexts (reviewed in detail in refs 24 and 25). Dissociation of NTF from CTF by an extracellular molecular partner unmasks a 15−25 amino acid tethered agonist that remains extracellularly on the N-terminus of CTF. Several studies have reported that NTF-truncated mutants of ADGRB2,[26] ADGRG1,[27] ADGRG2,[28,29,139] ADGRF1,[30] and ADGRE5[31] show constitutive activation of downstream signaling pathways. Further studies proved that this activity is due to the interaction of the tethered agonist with the cognate receptors. For instance, the deletion of tethered agonists in NTF-truncated ADGRD1,[32] ADGRG2,[139] and ADGRG6[32] abolished certain signaling pathways and exogenous addition of synthetic peptides, that are identical to tethered agonists, stimulated receptor signaling.[29,30,33,139] Alternative mechanisms of activation of aGPCRs exist. Unlike the aforementioned receptors, the interaction of NTF and CTF is required for proper signaling and function of ADGRC2 in the brain.[34] Moreover, circulating NTF of several aGPCRs have been reported[26,35,36] and the secreted NTF of ADGRB1 (Vasculostatin, a.k.a. Vstat120) showed antiangiogenic and antitumorigenic functions in glioma xenograft models,[37] pointing to CTF-independent roles that NTF may play in distant or neighboring cells. To add to the complexity of the aGPCR activation and signaling, recent studies have shown that while NTF and tethered agonist are required for certain signaling pathways of ADGRG1,[38] ADGRB1,[38] and ADGRG2,[139] they can be dispensable for interaction of these receptors with β-arrestins. Together, these studies suggest that GPS, NTF, CTF, tethered agonist, and other domains of aGPCRs play various functional roles.

AGPCRS IN CANCER

Multidomain NTF of aGPCRs enables cell−cell communication and cell-extracellular matrix interaction, processes that are dysregulated in cancer. Current evidence suggests that some aGPCRs regulate the cell cycle, proliferation, survival, and dissemination of cancer cells (Table 1).

For example, knockdown of ADGRL4 reduced the proliferation of glioblastoma cells *in vitro*.[151,152] ADGRG1 is upregulated in colorectal cancer tissues and cell lines and promotes tumor growth and metastasis via induction of epithelial to mesenchymal transition (EMT).[134] However, in melanoma cell lines, ADGRG1 suppressed the production of vascular endothelial growth factor, a known stimulator of tumor angiogenesis, and inversely correlated with melanoma progression in mouse tissues and xenograft models of human melanoma.[135] Antigrowth[153] and pro-metastasis[136] roles have also been reported for ADGRG1 in melanoma studies. ADGRB1 functions as an inhibitor of angiogenesis in pulmonary adenocarcinoma,[120] glioblastoma,[121] colorectal cancer,[123] and astrocytoma.[124] ADGRG2 showed functional roles in both benign (parathyroid adenoma[28]) and highly metastatic (Ewing sarcoma[142]) tumors. Various carcinoma-associated mutations (endometrial, lung, liver) in ADGRL1 revealed altered surface expression and exaggerated basal activity of the receptor.[154] In the era of -omics, there is now evidence of aberrant expression and mutational profile of aGPCRs in different malignancies that warrant future translational studies. Here, we review the current body of knowledge regarding the expression and function of aGPCRs in the five most common types of cancer (Figure 2).[155]

LUNG CANCER

Lung cancer is the leading cause of death and the most common cancer globally, totaling approximately 12% of new cancer cases in 2018.[156] ADGRB3 was shown to be one of the most significantly mutated genes in 13% of lung squamous and 5% of lung adenocarcinoma tumors.[157] These mutations span NTF, 7TM, and C-terminus of ADGRB3, and authors suggested that this protein might act as a putative tumor suppressor. This is in line with the reported antiangiogenic and antineurogenic activity of other members of this subfamily, ADGRB1 and ADGRB2.[158,159] Currently, small cell lung cancer (SCLC) and large cell neuroendocrine lung carcinomas (LCNEC) are differentiated based on morphological analysis, which tends to be a poor determinant of cancer subtype. Immunohistochemical (IHC) analysis of human lung tumors showed that ADGRB3 is expressed in the nucleus of a majority of SCLC samples but is either absent or expressed at low levels in the cytoplasm of LCNEC tissues.[129] The ability to use ADGRB3 staining to differentiate between SCLC and LCNEC will be of significant clinical importance. Nuclear localization and signaling of some GPCRs[160,161] and β-arrestins[162,163] have been reported in HEK293 cells and tumor cells. Interestingly, the NTF-truncated ADGRB3 was shown to interact with β-arrestin2.[164] Whether the nuclear ADGRB3 in SCLC cells is the activated form of the receptor that is transported to the nucleus by β-arrestin2 requires further studies.

MicroRNAs (miRNAs) are a family of noncoding small RNAs that regulate gene expression, are dysregulated in various cancers and are either tumor suppressors or oncogenes.[165] Down-regulation of miR-138-5p increased the expression of ADGRA2 in nonsmall-cell lung carcinoma

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Table 1. Comprehensive List of aGPCRs, Their Endogenous Ligands, Signaling Pathways, Altered Expression, and Mutation in Various Types of Cancer[a]

	receptor	ligand/extracellular partner	signaling pathways	cancer	expression/localization	mutation	disease significance
Subfamily I	ADGRL1 (LPHN1)	α-latrotoxin[39], Teneurin-2 (Lasso)[40,41], FLRT1, 2, 3[41,42], Neurexin-1α, -1β, -2β, -3β[43]	Gα_q, Gα_o[44]	AML[45], Lung, Liver, Endometrial carcinoma[154]	decreased[45]	-	Overexpression of ABCB1 drug transporter[45]
	ADGRL2 (LPHN2)	FLRT3[42]	-	Breast[46], Squamous Cell Lung Carcinoma[47]	increased[46,47]	Q693H[47]	Escape immune surveillance by activating the Tim-3-Galectin-9 pathway[46]
	ADGRL3 (LPHN3)	FLRT1, 2, 3[42,48], Teneurin-3[42]	-	Breast[49]	increased[49]	-	Correlated with axillary lymph node metastasis[49]
	ADGRL4 (ELTD1)	-	-	Renal[50], Colorectal[50], HNSCC[50], Ovarian[50], Glioblastoma[151,152]	increased[50]	-	Correlated with a positive prognosis in various cancers by regulating angiogenesis and vessel maturation[50]
Subfamily II	ADGRE1 (EMR1)	-	-	CRC[51], AML[52]	increased[51,52]	-	Azoxymethane mouse models of CRC show increased expression of ADGRE1[51]
	ADGRE2 (EMR2)	Chondroitin Sulfate B[53]	Gα_{16}[54]	Lung[55,56] AML[52], Glioblastoma[57], Breast[58]	increased[52,55,58], localization[20]	-	Decreases adhesion and increases proliferation in lung cancer[55] Correlated with EMT markers[56] Associated with a more invasive phenotype[57,58] Cytoplasmic expression correlated with worse prognosis[58]
	ADGRE3 (EMR3)	-	-	Relapsed CRC[59,60], HCC[61], Glioblastoma[62]	increased[59-62]	-	Hypomethylated at CpG islands[61] Increase in invasive phenotype[62]
	ADGRE4 (EMR4)	-	-	-	-	-	
	ADGRE5 (CD97)	CD55[63], α5β1 integrin[20], Chondroitin Sulfate B[53], CD90[64]	G$\alpha_{12/13}$[31]	CRC[65-68], Rectal, Adenocarcinoma[69], Pancreatic[70], IHC[71], Gastric[72], Breast[73], HCC [74], Esophageal [75], Glioblastoma[76], Ovarian[77], Gallbladder[78], AML[79,80], OSCC[81], Thyroid[82], Prostate[31]	increased[65,69-71,73,74,76-82], localization[67]	-	Poor clinical outcome and increased invasion[66,68-70,73,76-83] Cytoplasmic ADGRE5 correlates with more malignant phenotype[67] Expressed only on the invasive front of rectal tumor cells[69] Biliary soluble ADGRE5 is a negative prognostic biomarker in IHC[71] Exosome-mediated proliferation [71] Correlated with tumor metastasis[74] Hypomethylated promoter[75]
Subfamily III	ADGRA1 (GPR123)	-	-	-	-	-	
	ADGRA2 (GPR124)	Glycosaminoglycans[84], WNT7[85]	β-catenin[85]	Glioblastoma[86], Osteosarcoma[87], Urothelial Carcinoma[88], Lung[166]	increased and decreased[86], increased[88]	-	Alteration in ADGRA2 expression leads to altered microtubule dynamics during mitosis leading to chromosomal instability[86] Contributes to tumor angiogenesis[87]
	ADGRA3 (GPR125)	-	-	CRC[89]	increased[89]	-	Correlates with increased metastasis and worse prognosis[89]
Subfamily IV	ADGRC1 (CELSR1)	-	Rho Kinase[90]	-	-	-	
	ADGRC2 (CELSR2)	-	Ca^{2+}[91]	Breast[92]	localization[92]	-	Cytoplasmic localization is a negative prognostic marker in HER2+ breast carcinoma[92]
	ADGRC3 (CELSR3)	-	Ca^{2+}[91]	OSCC[93], AML[52], HCC[94], HNSCC[95]	decreased[52,93], increased[94,95]	-	Highly methylated in oral carcinomas[93] Increased expression correlated with poor prognosis in HCC[94] Decreased expression is correlated with better survival in HNSCC[95]
Subfamily V	ADGRD1 (GPR133)	-	Gα_s[96]	Glioblastoma[97], AML[98], Gastric[99]	increased[97,98], decreased[99]	-	Protumorigenic role in hypoxic glioblastoma tumors[97] Associated with poor clinical outcome[98] Downregulated by lncRNA and miRNAs in gastric cancer[99]
	ADGRD2 (GPR144)	-	-	Lung[100], HNSCC[95]	increased[95]	-	Frequently mutated due to benzo(a)pyrene exposure[100] Increased expression is correlated with better survival in HNSCC[95]

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Table 1. continued

	receptor	ligand/extracellular partner	signaling pathways	cancer	expression/localization	mutation	disease significance
Subfamily VI	ADGRF1 (GPR110)	Synaptamide[101]	$G\alpha_s$[101], $G\alpha_q$[30]	Lung[102-104], Prostate[102], Breast[105], ALL[106], Osteosarcoma[107], HCC[108], Thyroid[109]	increased[102,104-107]	-	Various polymorphisms associated with NSCLC susceptibility[103] Knockdown decreased anchorage-independent growth in BT747 breast cancer cells[105] Prostate cancer lines express splice-variants of ADGRF1 [102] Knockdown decreased proliferation and its expression served as a prognostic marker for osteosarcoma[107] Genomic deletion of ADGRF1 decreased the hepatocellular carcinogenesis[108] Expression can be used to distinguish papillary thyroid cancer from other thyroid malignancies[109]
	ADGRF2 (GPR111)	-	-	-	-	-	-
	ADGRF3 (GPR113)	-	-	SBNET[110,111]	increased[110,111]	-	Increased expression in the primary SBNET and secondary lesions[110,111]
	ADGRF4 (GPR115)	-	$G\alpha_{15}$[112]	Lung[113]	increased[113]	-	Associated with TRIMP58 methylation in lung squamous cell carcinoma, may be a prognostic marker [113]
	ADGRF5 (GPR116)	Surfactant Protein D[114]	$G\alpha_q$[115]	CRC[116], Breast[115], Gastric[117]	increased[115,116]	G2731C[117], G2276A[117]	Patients with high levels of ADGRF5 showed distant metastasis and poor tumor differentiation[116] Expression promotes migration, invasion and metastasis[115]
Subfamily VII	ADGRB1 (BAI1)	Phosphatidylserine[118], Matrix metalloprotease 14[119]	$G\alpha_{12/13}$[38], β-Arrestin[38]	Lung[120], Glioblastoma[121], CRC[122,123], Astrocytoma[124], Breast[125]	decreased[84,125]	-	Functions as an inhibitor of angiogenesis[120,121,123,124] Decrease in expression correlates with a favorable prognosis[125] Ectopic expression of NTF decreased metastasis to the brain[125]
	ADGRB2 (BAI2)	Glutaminase Interacting Protein (GIP)[126], Furin[26]	$G\alpha_{16}$[26]	CRC[127]	increased[127]	-	Correlates with tumorigenesis and tumor growth [127]
	ADGRB3 (BAI3)	C1q-like proteins[128]	-	Lung[129], Glioma[130]	increased[129], decreased[130]	-	Expression is increased in SCLC and can be used to differentiate from LCNEC[129]
Subfamily VIII	ADGRG1 (GPR56)	Collagen III [96],Transglutaminase II[21,131]	$G\alpha_{q/11}$[132], $G\alpha_{12/13}$[133], β-Arrestin[38]	CRC[134], Melanoma[135,136], Prostate[137], AML[138]	increased[134,135,137,138]	-	Promotes metastasis by inducing EMT[134,136,137] Suppresses the expression of VEGF[135] Increased expression can be used to differentiate leukemic stem cells[138]
	ADGRG2 (GPR64)	-	$G\alpha_q$[139,140], $G\alpha_s$[28], $G\alpha_{13}$[139], β-Arrestin[139,140]	Breast[141,142], ES[142], Parathyroid[28], Endometrial [143]	increased[28,141] decreased[143]	-	Knockdown of ADGRG2 decreased cell migration[141] Promotes invasiveness and metastasis[142] Regulates PTH release by parathyroid adenoma[28] Functions as a tumor suppressor in endometrial cancer[143]
	ADGRG3 (GPR97)	Beclomethasone Dipropionate[112]	$G\alpha_o$[112]	-	-	-	-
	ADGRG4 (GPR112)	-		NEC[144]	increased[144]	-	A novel marker for NEC cells[144]
	ADGRG5 (GPR114)	-	$G\alpha_s$[112]	AML[52]	increased[52]	-	Consistently upregulated in AML[52]
	ADGRG6 (GPR126)	Collagen IV[22], Laminin-211[145]	$G\alpha_s$ [32]	Bladder[146]	intronic mutations[146]	multiple intronic mutations[146]	Mutational burden can be a prognostic marker in bladder cancer[146]
	ADGRG7 (GPR128)	-	-	Lymphoma[147]	gene fusion[147]	-	ADGRG7 fused with TRK fused gene (TFG) in lymphoma tumors[147]
Subfamily IX	ADGRV1 (GPR98)	-	$G\alpha_i$[148], $G\alpha_s$[149], $G\alpha_q$[149]	CRC[150]	increased[150]	-	Inhibition by miR-145 sensitizes cells to Oxaliplatin[150]

[a]Notation: ALL, acute lymphoblastic leukemia; AML, acute myelocytic leukemia; CRC, colorectal carcinoma; ES, Ewing's sarcoma; HCC, hepatocellular carcinoma; HNSCC, head and neck squamous cell carcinoma; IHC, intrahepatic cholangiocarcinoma; NEC, neuroendocrine carcinoma; OSCC, oral squamous cell carcinoma; SBNET, small bowel neuroendocrine tumors.

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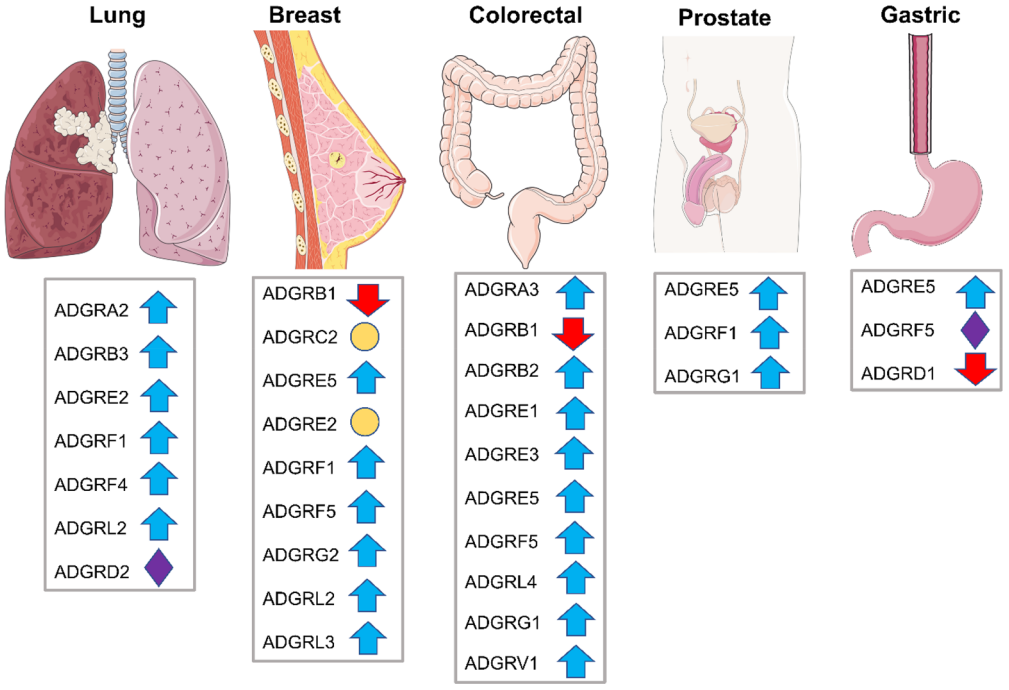

Figure 2. List of aGPCRs with altered expression (blue and red arrows), mutation (purple diamonds), or localization (orange circles) in the five most common cancers globally (organ images are taken from https://smart.servier.com).

(NSCLC) cell lines and patient-derived cells.[166] NSCLC patients who are treated with gefitinib, a common tyrosine kinase inhibitor, often become resistant to this drug.[167] Interestingly, introducing miR-138-5p to resistant NSCLC cells down-regulated ADGRA2 and resensitized cells to gefitinib.[166] Although the endogenous ligand of ADGRA2 is unknown, this receptor has been implicated in tumor angiogenesis, a known mechanism of gefitinib resistance in lung cancer patients.[166−169] Whether inhibition of ADGRA2 by a small molecule or biologic can resensitize patients to gefitinib is yet to be explored.

Expression profiling revealed that 97 miRNAs were differentially expressed in NSCLC patients' lungs compared with normal lung tissues, of which miR-099a was one of the most down-regulated miRNAs in NSCLC tissues. Expression of miR-099a in NCI-H1650, NCI-H1975, and NCI-H1299 lung adenocarcinoma cell lines reduced expression of ADGRE2 and increased cell cycle arrest and apoptosis.[55] Rescue experiments suggested that ADGRE2, a target of miR-099a, mediates NSCLC cell migration and its knockdown increases adhesion and decreases proliferation. ADGRE2 expression also correlated with β-catenin expression,[55] a known marker for EMT and metastasis in lung adenocarcinoma.[56] IHC analysis of 119 lung cancer patient biopsies revealed that ADGRE2 is upregulated in approximately 12% of cases.[55] ADGRE2 binds to chondroitin sulfate,[53] a proteoglycan that is involved in lung growth[170] and is present at elevated levels in lung tumors.[171] This evidence, combined with the fact that ADGRE2 couples to Gα15,[112] a promiscuous Gα protein that activates phospholipase Cβ, provides strong grounds for the screening of small molecules that interfere with ADGRE2-mediated signaling and migration of NSCLC cells.

Insertional mutagenesis experiments, either by retroviruses or lentiviruses, have been exploited as a tool to identify genes that potentially regulate cell growth and culminate in tumorigenesis.[172] Genomic localization of proviral sequences after a retroviral screen in mice suggested that ADGRF1 is a proto-oncogene in mouse leukemia,[102] which was corroborated by additional reported insertion sites.[173,174] Lum et al. followed this proto-oncogenic indication by mRNA and protein expression analysis and found that, whereas ADGRF1 expression was low in lung cancer cell lines, lung adenocarcinoma tumor samples showed upregulated ADGRF1 compared to either normal lung, squamous, or small lung tumor samples.[102]

BREAST CANCER

Breast cancer was the second most commonly diagnosed cancer in 2018 with over 2 000 000 newly diagnosed cases.[156] Several aGPCRs show altered expression or mutation in breast malignancies (Table 1).[73,92,105,175] ADGRC2 was initially shown to be down-regulated in human epidermal growth factor receptor 2 (HER2)-positive breast carcinomas.[176] Further immunohistochemical studies by the same group did not identify a significant correlation between ADGRC2 expression and either HER2 or estrogen receptor (ER) status of breast tumor tissues or cell lines.[175] However, they identified a small group of cell lines and tumors that show striking down-regulation of ADGRC2, pointing to a potential impact of this receptor in a subset of breast cancers. ADGRC2 is a member of the nonclassical cadherin family of proteins due to the presence of several cadherin domains in its NTF. Interestingly, cadherins are involved in cell−cell communication and cell adhesion, and E-cadherin promotes metastasis in diverse models of invasive ductal carcinomas.[177−179] Therefore, it would be interesting to know whether the deletion of cadherin domains in ADGRC2 changes the metastatic potential of breast cancer cells.

Localization and expression of ADGRE2 correlated with breast cancer patient prognosis.[58] While ADGRE2 is not expressed in normal breast epithelial cells, invasive breast carcinomas and ductal carcinoma in situ (DCIS) showed

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upregulation of ADGRE2.[58] Nuclear expression of ADGRE2 was correlated with lower tumor grades and a longer disease-free survival.[58] Since inactive GPCRs do not reside in the nucleus, it is possible that ADGRE2 is either activated on the cell surface and endocytosed to the nucleus or it is not shuttled to the plasma membrane after translation. Further research is necessary to define the mechanism by which ADRGE2 regulates breast cancer cell function and to confirm nuclear localization as a prognostic biomarker.

Data from the Cancer Genome Atlas (TCGA) show that 52% of patients with invasive ductal carcinoma have reduced levels of ADGRB1, which correlates inversely with patient survival.[125] This is consistent with the down-regulation of ADGRB1 in several other tumors including glioblastoma,[121] colorectal,[123] and lung cancer.[120] The secreted N-terminal fragment of ADGRB1 (Vasculostatin, a.k.a. Vstat120) was shown to suppress growth in xenograft models of glial tumors.[37] Vstat120 contains an arginine−glycine−aspartate domain and five thrombospondin type-1 repeats, motifs that are known modulators of angiogenesis.[180,181] Overexpression and consequent secretion of Vstat120 reduced the viability of various subtypes of breast cancer cell lines.[125] Injection of Vstat120-expressing virus into the brain of breast cancer-derived brain metastases (BCBM) mouse models significantly decreased the tumor size and disease burden and increased survival.[125] This experiment is of significant clinical importance because BCBM is a feature of treatment-resistant HER2-positive and triple-negative breast cancer, for which the standard of care is systemic chemotherapy and radiation with poor prognosis and low survival rates.[182] Given the antiangiogenic effects of Vstat120 and its motifs, it would be important to investigate their stability and bioavailability in mouse models of cancer. Also, the therapeutic efficacy of these molecules in combination with other current therapies warrants future research.

ADGRE5 is upregulated in MDA-MB231, MDA-468, MCF-7, and T47D breast cancer cell lines and its knockdown decreased cell growth, proliferation, and migration.[73] However, the mechanisms by which ADGRE5 regulates these cellular functions in breast cancer cell lines are mainly unknown. Independent studies have provided contradictory results whether the expression of the endogenous ligand of ADGRE5, CD55, correlates with breast cancer prognosis positively or negatively.[183,184] This might be due to the different methods used by authors to define "high and low expression". It is noteworthy that ADGRE5[73] and CD55[184] are coexpressed on the surface of MCF-7 cells. Further studies may reveal whether this receptor−ligand pair are colocalized in breast tumor tissues as well and if deletion of either or both proteins alters the *in vivo* manifestation of breast tumor. As elaborated in more detail later in this minireview, the expression of ADGRE5 in various epithelial carcinomas correlates with the stage and progression of the tumor.

As great strides are made in cancer treatments, there are still many patients who develop resistance to targeted therapies. Bhat et al. recently showed that several aGPCRs are expressed in cancer stem cells and anti-HER2 therapy-resistant cells. Using Aldefluor, a nonimmunological fluorescent marker for stemness, Baht et al. found that ADGRB3, ADGRE2, ADGRA2, ADGRF5, and ADGRF1 are all overexpressed in cancer stem cells.[105] The only aGPCR found to be expressed in both cancer stem cells and anti-HER2 therapy-resistant cell lines was ADGRF1.[105] Knockdown of ADGRF1 in BT747 cells decreased anchorage-independent growth, a common feature of metastatic cell lines and reduced the mammosphere formation, suggesting a role for ADGRF1 in cancer stemness.[105] These data warrant further investigation into the downstream effects and potential targeting of ADGRF5 in HER2+ breast cancer.

Knockdown of ADGRF5 in highly metastatic breast cancer cell line, MDA-MB-231 reduced the cell migration *in vitro* and metastasis in mammary tumor mouse models *in vivo*.[115] The potential role of ADGRF5 in cell invasion was further confirmed by the ectopic expression of the receptor in less-metastatic breast cancer lines (MCF-7 and Hs578T).[115] Activation of a well-known cytoskeletal remodeling signaling cascade, Gαq, p63RhoGEF and small GTPases, RhoA and Rac1 was confirmed as the potential mechanism of cell motility by ADGRF5 in breast cancer cells.[115] The increased expression of ADGRF5 in human breast cancer tissues correlated with cancer metastasis and poor prognosis,[115] further suggesting this receptor as a potential candidate for breast cancer therapy.

The expression of ADGRG2 in breast cancer cell lines has been debated. Richter et al. showed low to no expression of ADGRG2 transcripts in MDA-MB-231 and Hs578T breast cancer cell lines.[142] We have also not been able to show the expression of ADGRG2 in MDA-MB231 cells at either mRNA or protein level (data not shown). However, Peeters et al. revealed the effect of ADGRG2 knockdown on migration and adhesion of these cell lines, presumably via its effect on RelB, a member of the NF-κB family.[141] Surprisingly, Peeters et al. did not provide expression data for ADGRG2 at either mRNA or protein level in either cell lines. An impedance-based assay (xCELLigence) showed that ADGRG2 knockdown delays breast cancer cell adhesion but does not modulate cell proliferation.[141] Constitutive activation of the serum response element (SRE) transcription factor was dependent on the autoproteolysis of ADGRG2 at its GPS site when the receptor was overexpressed in HEK293 cells.[141] Unlike reports on the inhibitory function of NTF in ADGRG2 signaling,[28,29,139] Peeters et al. showed that NTF plays a crucial role in the activation of both NF-κB and SRE pathways by ADGRG2.[141] Further studies to profile the expression and localization of ADGRG2 in breast cancer cell lines and patient breast tumor-derived cells are necessary to provide a thorough understanding of its function in this disease.

COLORECTAL CANCER

The current standard of care for colorectal cancer (CRC), the third most commonly diagnosed cancer,[155] is surgical resection, radiotherapy, and chemotherapy.[185] In recent years targeted therapies such as inhibitors of angiogenesis, immune checkpoint, and epidermal growth factor receptor (EGFR) have turned CRC into a highly treatable disease. However, resistance due to tumor mutation and recurrence following surgery warrant further studies.[186]

The expression of several aGPCRs is changed in CRC. While ADGRB1 is down-regulated in the colon mucosa of CRC patients,[122] ADGRB2 is upregulated in advanced CRC[127] and ADGRE3 is upregulated in CRC biopsies of relapsed patients compared with patients who are disease-free.[51,59,60] In addition, expression of ADGRE1 is decreased in colon tissue biopsies of mouse models of colorectal carcinoma compared with control mice.[51] Unfortunately, these aGPCRs have no prognostic indications and no further work has been done to elucidate their mechanistic roles in CRC.

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Other aGPCRs have shown more promise in the laboratory and clinical settings. Analysis of three Gene Expression Omnibus (GEO) data sets and one data set from TCGA (151 cases of CRC) revealed that ADGRA3 is down-regulated in 78% of CRC specimens and its elevated expression in 22% of the samples is associated with prolonged recurrence-free survival.[89] CRC patients with upregulated ADGRA3 showed reduced KRAS mutation, smaller tumors, and less metastasis.[89] Interestingly, gain-of-function mutations in KRAS contribute to the transition from adenoma to CRC[187] and are good predictors of resistance to EGFR therapy.[186] Overexpression of ADGRA3 in a CRC cell line (HCT116) suppressed the Wnt/β-catenin signaling pathway, a known driver of CRC[89,188] and down-regulated c-Myc and cyclin-D1. There is compelling evidence that KRAS mutations can cause aberrant Wnt/β-catenin signaling, which will cause an oncogenic transformation in intestinal epithelial cells.[189] Yet, it is unclear whether there is a crosstalk between ADGRA3 and KRAS at the levels of Wnt/β-catenin signaling.

High ADGRE5-expressing CRC cells show greater localization of β-catenin in the nucleus, indicating activation of the Wnt/β-catenin signaling pathway.[65,68] Wobus et al. found that CRC cells with increased expression of ADGRE5 showed an elevated invasion and a poor clinical outcome.[65,68,190] Given that ADGRE5 has an observable effect on tumor cell invasion, migration, and secondary metastasis in various cancers,[31,74,191,192] its interactions with junctional proteins were studied. Proximity ligation and coimmunoprecipitation assays in various human CRC cell lines showed a strong interaction among ADGRE5, β-catenin, and E-cadherin when compared to normal tissues and cell lines.[67] Malignant samples had reduced membrane-bound and increased cytoplasmic ADGRE5.[67] These results suggest that ADGRE5 plays an important role in the regulation of cell junctions in normal colon tissue. Whether the cytoplasmic ADGRE5 is an indication of its prior activation and endocytosis in CRC cells awaits further investigation. IHC staining of various rectal adenocarcinoma tissue samples showed a strong coexpression of ADGRE5 and CD55 in the invasive front of the tumor.[69] However, cells in the center of the tumor showed little to no expression of either ADGRE5 or CD55. Patients with high ADGRE5 expression showed a less favorable prognosis, more metastatic burden, and a higher rate of clinical recurrence.[69] These data indicate that ADGRE5 and its ligand, CD55, may have a prognostic role as a biomarker in CRC.

Protein and mRNA analysis of 48 colorectal carcinoma cases showed a significant increase in ADGRF5 levels when compared with normal tissues.[116] These results were confirmed in three microarrays from the Oncomine database and IHC staining of over 90 CRC samples.[116] Patients expressing high levels of ADGRF5 showed an increase in distant metastasis and histological differentiation.[116] Univariate analysis of these results, along with several other in-silico studies, show that high levels of ADGRF5 could act as an unfavorable prognostic indicator in CRC patients. miR-511−5p is known to be down-regulated in a variety of CRC cell lines, and patients who expressed elevated levels of miR-511-5p show higher survival rates.[193] *In vitro* overexpression of miR-511-5p mimetics in CRC cells reduced proliferation and colony formation and increased cell apoptosis.[193] Interestingly, miR-511-5p binds the 3′UTR of the *ADGRF5* gene to repress its transcription, and overexpression of ADGRF5 reverses the antitumor features of miR-511-5p.[193] Together, these data support tumorigenic roles for ADGRF5.

mRNA, IHC, and *in situ* hybridization analyses showed that ADGRG1 is highly expressed in CRC specimens[194] and colonic crypt cells[195] compared with normal gastrointestinal tissues and cells. This overexpression is intensified in mice that express progastrin, a peptide that is upregulated in CRC and other cancer cell lines.[195,196] Jin et al. found that ADGRG1 directly interacts with progastrin to increase the proliferation rate of colonic cells and the genomic deletion of ADGRG1 increases apoptosis in the colonic mucosa and decreases proliferation in mice.[195] The increased expression of ADGRG1 predicted a worse prognosis for patients suffering from CRC[134] and knockdown of ADGRG1 down-regulated mesenchymal markers, N-cadherin, and vimentin via the PI3K/AKT pathway.[134] The current screening method for CRC is an optical colonoscopy that does not detect the early stages of cancer development. It remains to be investigated whether ADGRG1 can potentially be a less invasive diagnostic biomarker for CRC.

PROSTATE CANCER

There were 1 276 106 newly diagnosed cases of prostate cancer in 2018, globally. Because of the indolent nature and slow progression of prostate cancer many cases remain undiagnosed until later stages of the disease. Examination of prostate cancer screenings such as prostate-specific antigen and digital rectal exam lack internal validity and have shown inconsistent results and false-positives.[197]

Histological analysis of a prostate tissue array derived from 36 adenocarcinoma cases revealed that ADGRE5 is upregulated in these tumors compared with normal adjacent tissues.[31] This was corroborated by the high expression of ADGRE5 in some prostate cancer cell lines (PC3 and DU145) and the low expression in nontransformed prostate cells. The depletion of ADGRE5 in DU145 cells reduced the serum-induced activation of RhoA small GTPase *in vitro* and cell migration and invasion in Matrigel.[31] The described mechanism of ADGRE5-mediated migration of prostate cells is consistent with a previous study, in which ADGRE5 regulated the migration of neural progenitor cells through Gα12/13 G proteins and RhoA small GTPase.[133] Mice injected with ADGRE5-depleted PC3 cells showed a significant reduction in bone metastasis but no change in tumor growth when compared with mice that were injected with parental PC3 cells.[31] In addition to ADGRE5, the increased expression of lysophosphatidic acid receptor 1 (LPAR1) has been reported in prostate cancer cells.[198] Interestingly, the ectopic coexpression of ADGRE5 and LPAR1 in LNCaP cells (an androgen-sensitive prostate adenocarcinoma) revealed that these GPCRs heteromerize and ADGRE5 potentiates the LPA-induced RhoA activation.[31] Heteromerization and crosstalk of various GPCRs and the consequent regulation of tumorigenesis and metastasis of prostate,[199,200] breast,[199,200] and glioblastoma[201] cells have been previously reported. Histological examination indicated an association between expression of LPAR1 and ADGRE5 in prostate cancer biopsies.[31] Together, these data suggest crosstalk between LPAR1 and ADGRE5 in prostate tumor cells. Considering that LPAR1 antagonists have not yet been approved to mitigate tumor burdens and metastasis, it would be interesting to examine whether inhibition of ADGRE5 by small molecules or specific antibodies suppresses the LPAR1-mediated metastasis.

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Prostate biopsies from old subjects, which are prone to benign hyperplasia or have undiagnosed cancer, showed higher expression of ADGRF1.[102] There is a spectrum of ADGRF1 expression among main prostate cancer cell lines; high in PC3, low in LNCaP, and negative in DU145. Histological analysis with antibodies raised against two distinct peptides from the NTF of ADGRF1 showed differential staining in prostate adenocarcinoma tissues, suggesting potential expression of different splice variants.[102] Such differential staining precluded a comparative analysis of ADGRF1 expression between benign prostate hyperplasia and prostate adenocarcinomas and emphasized the importance of relative quantification of splice variants of aGPCRs in tumors.

GASTRIC CANCER

Gastric cancers are the fifth most commonly diagnosed cancer in the world.[156] Aust et al. found that ADGRE5 was expressed in 44 of 50 gastric cancer biopsies.[202] Alternative splicing generates three isoforms of ADGRE5 that contain three, four, or five repeats of EGF domains on their NTF. Overexpression of the smaller isoform, ADGRE5/EGF1,2,5 in BGC-823 stomach adenocarcinoma cells increased their invasive behavior *in vitro*.[203] In line with these findings, orthotopic mouse models of gastric carcinoma that lacked the ADGRE5/EGF1,2,5 showed reduced metastatic spread and tumor volume.[192] However, the full-length isoform, ADGRE5/EGF1,2,3,4,5 suppressed the invasion and increased proliferation. These studies indicate that the characteristics of gastric tumor cells may be regulated by the balance of ADGRE5 splice variants.[203]

Recently, Chao et al. found that the exosomes isolated from the stomach adenocarcinoma cell lines that express wild-type ADGRE5 stimulated migration of other cells in a transwell assay.[72] This was accompanied by phosphorylation of the major signaling molecules of the MAPK pathway. Consistent with these findings, exosomes released from ADGRE5-expressing tumors increased metastasis of gastric adenocarcinomas.[204] In a footpad mouse model of aggressive gastric adenocarcinoma, Liu et al. found that tumors lacking ADGRE5 show a diminished metastasis and metastatic niche formation.[204] Exosomes isolated from SGC-L, an SGC-7901 cell-derived highly metastatic gastric cancer cell line expressing ADGRE5, were also able to increase the metastatic phenotype of tumor cells.[204] Taken together, these studies suggest that ADGRE5 increases cell proliferation and metastasis in gastric cancer via vesicle-mediated tumor cell communication and activation of the MAPK pathway.

A recent study showed that ADGRF1 mRNA and protein are significantly upregulated in tumor biopsies compared with paired adjacent normal tissues resected from 117 gastric cancer patients.[205] Patients with high ADGRF1 protein levels had shorter survival and increased recurrence after surgery compared with gastric cancer patients with low ADGRF1 expression. These data suggest that ADGRF1 may be a candidate biomarker for diagnostic purposes in gastric cancer patients. *N*-Docosahexaenoylethanolamine (synaptamide, *a.k.a.* DHEA), a stimulant of neurite growth, was recently shown to induce cyclic AMP production via ADGRF1.[101] It would be interesting to know (a) whether the level of synaptamide, an endogenous metabolite ligand of ADGRF1, is altered in the gastric tumor microenvironment, (b) what the pathologic impact of synaptamide-ADGRF1 interaction is, and (c) what molecular mechanism(s) are used by ADGRF1 in gastric cancer cells.

CONCLUSIONS AND POTENTIAL THERAPEUTIC APPROACHES

Although the field of aGPCR research has seen constant growth in terms of engaged signaling pathways in the past decade, the physiological functions of these receptors are yet to be explored further. In particular, their role in all aspects of cancer, from tumor initiation to metastasis is incompletely understood. aGPCRs are implicated in diverse diseases from diabetes to various neoplasms. However, there are currently no approved drugs targeting any aGPCRs.

The difficulty in obtaining structural information from aGPCRs has hampered the process of developing small molecules or biologics to target them. On the other hand, the large NTF and its multiple domains provide potential sites to target therapeutically. The recent discovery of an ADGRG1 antagonist[206] gives hope for the development of future small molecules with proper pharmacology to regulate the function of these understudied receptors in cancer. In addition to small molecules, antibodies against domains of the NTF can be potentially interesting modulators. Salzman et al. showed that monobodies designed for certain domains on the NTF of ADGRG1 can act as activators or inhibitors of G protein signaling.[207] It remains to be explored whether antibodies against either tethered agonist or its binding site(s) will act as antagonists.

Another hypothetical approach that may modulate aGPCR function is the design of cell-permeable inhibitors of autoproteolysis, as this cleavage is required for activation of certain signaling pathways by ADGRG2 and ADGRG1.[141,208]

aGPCRs interact strongly with β-arrestins, particularly in the absence of NTF.[38,139] The signaling bias of classical GPCRs toward either G protein or β-arrestin pathways and their physiological effects have challenged the drug development.[209−211] This phenomenon should be taken into account in the development of aGPCR modulators too.

Fibrosis in the tumor microenvironment, a side effect of protease actions and metastasis, alters the composition of ECM dramatically.[212] Given the ECM-binding domains on the NTF of some aGPCRs, it would be interesting to examine whether these changes modulate aGPCR activity and thereby proliferation and migration of tumor cells. Also, targeting the large ECM ligands of aGPCRs to interfere with the protein−protein interaction can be a potential approach to regulate aGPCR functions in cancer.

On average, aGPCRs have 19 transcript variants with tissue-dependent expression patterns, leading to functional differences.[213] As mentioned above for ADGRF1 in prostate cancer and for ADGRE5 in gastric tumors, splice variants of aGPCRs show a differential impact on tumorigenesis. Therefore, it is crucial to use the genomics/bioinformatics tools such as RNA-Seq to quantify various transcripts of aGPCRs of interest in tumor specimens at the first stages of cancer studies.

The body of evidence provided here points to fundamental roles that aGPCRs may play in promotion or prevention of cancer, and we hope this would trigger future translational studies to explore their potential as therapeutic targets.

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AUTHOR INFORMATION

Corresponding Author

Nariman Balenga − *Division of General & Oncologic Surgery, Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland 20201, United States; Molecular and Structural Biology program at University of Maryland Marlene and Stewart Greenebaum Comprehensive Cancer Center, Baltimore, Maryland 20201, United States;* orcid.org/0000-0002-2741-9595; Phone: 410-706-3261; Email: nbalenga@som.umaryland.edu; Fax: 410-706-3260

Author

Abanoub A. Gad − *Graduate Program in Life Sciences, University of Maryland, Baltimore, Maryland 20201, United States; Division of General & Oncologic Surgery, Department of Surgery, University of Maryland School of Medicine, Baltimore, Maryland 20201, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsptsci.9b00093

Notes

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