Review Article

Strength and duration of GIPC-dependent signaling networks as determinants in cancer ☆

Tasmia Ahmed [a]; **Karthikeyan Mythreye** [d];
Nam Y. Lee [a,b,c,1,*]

[a] Deparment of Pharmacology, College of Medicine, University of Arizona, Tucson, AZ, USA
[b] Department of Chemistry and Biochemistry, University of Arizona, Tucson, AZ, USA
[c] The University of Arizona Cancer Center, Tucson, AZ, USA
[d] Division of Molecular and Cellular Pathology, University of Alabama Birmingham, Birmingham, AL, USA

Abstract

GIPC is a PDZ-domain containing adaptor protein that regulates the cell surface expression and endocytic trafficking of numerous transmembrane receptors and signaling complexes. Interactions with over 50 proteins have been reported to date including VEGFR, insulin-like growth factor-1 receptor (IGF-1R), GPCRs, and APPL, many of which have essential roles in neuronal and cardiovascular development. In cancer, a major subset of GIPC-binding receptors and cytoplasmic effectors have been shown to promote tumorigenesis or metastatic progression, while other subsets have demonstrated strong tumor-suppressive effects. Given that these diverse pathways are widespread in normal tissues and human malignancies, precisely how these opposing signals are integrated and regulated within the same tumor setting likely depend on the strength and duration of their interactions with GIPC. This review highlights the major pathways and divergent mechanisms of GIPC signaling in various cancers and provide a rationale for emerging GIPC-targeted cancer therapies.

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Introduction

PDZ domains represent one of the most common protein-protein interaction domains present in all organisms from bacteria to human [1–5]. GAIP interacting protein C-terminus, GIPC/GIPC1, also known as synectin [6], is a key member of this family [1] that functions as an essential trafficking adaptor for membrane receptors, signaling effectors, and protein complexes- altogether comprising more than 50 protein-protein interactions to date [7]. As such, GIPC is considered a highly versatile molecule that controls vastly diverse cellular and pathophysiological processes.

In more recent years, GIPC has gained increasing attention for its roles in cancer. It not only serves as an antigen in breast and ovarian cancer [8–10] but has been strongly implicated in the progression of numerous malignancies including pancreatic [11,12], colon [13,14], skin [15], epidermal cancer stem (ECS) cell [17], glioma [18], lung [19], and gastric cancers [20] by potentiating tumor growth, invasion, metastasis, and cell survival. In contrast, GIPC is downregulated in cervical cancer [21], primary kidney tumor [20], primary colorectal tumor [20], and primary prostate cancer [20] and may promote tumor suppressive effects in these settings. These opposing effects likely arise from varying signaling contexts, the type of PDZ ligand, and the overall strength with which it binds in various cancer cells.

In this review, we discuss the major functions of GIPC in different cancers and their cellular mechanisms by focusing on its ability to differentially interact with membrane receptors, signaling complexes and oncoproteins. We also highlight the emerging roles of GIPC as a potential therapeutic target in cancer therapy.

Intracellular Signaling and Trafficking Functions of GIPC

GIPC was initially identified as an interacting partner of the GTPase-activating protein RGS-GAIP for G-protein coupled receptor subunit Gαi [1]. Although the GIPC PDZ domain preferentially interacts with
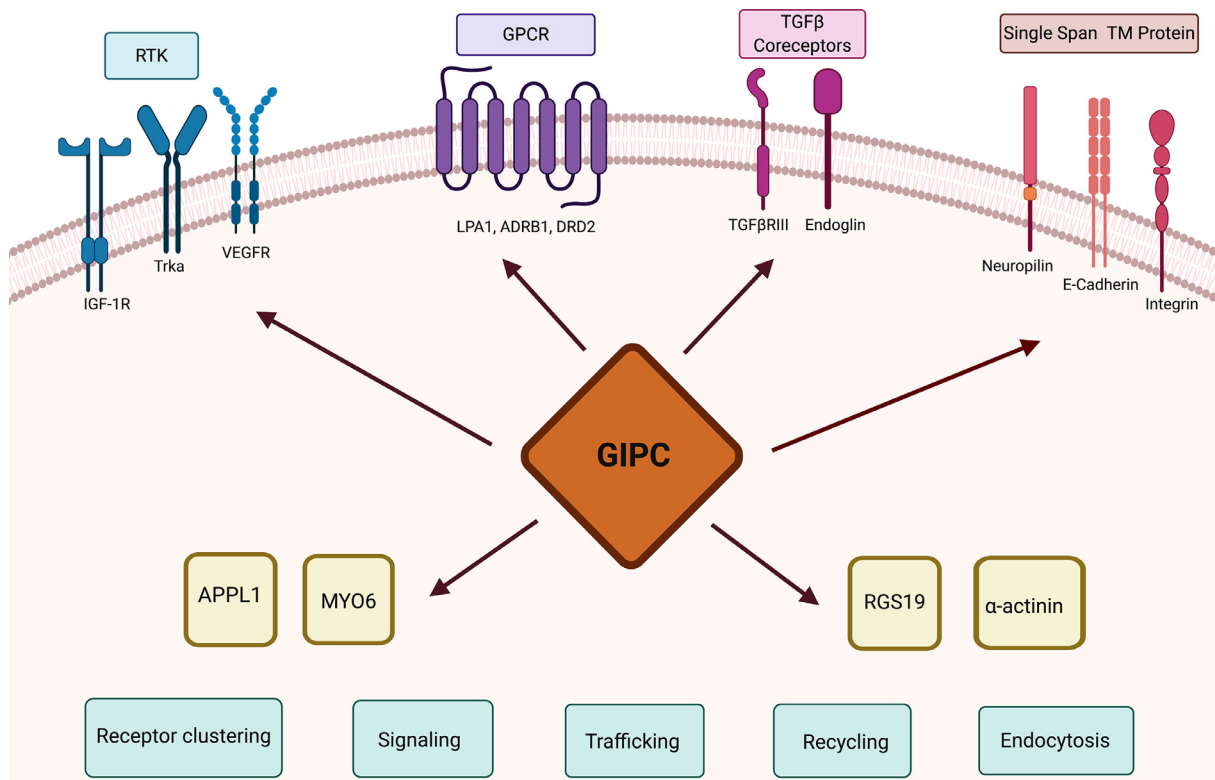
Figure 1. GIPC-dependent signaling complexes. GIPC interacts with various transmembrane receptors to promote their cell surface retention (e.g., TβRIII and endoglin) or internalization for enhanced endosomal signaling (e.g., IGF1R). GIPC also interacts with cytoplasmic signaling and trafficking proteins (e.g., Myo6).

the consensus class I PDZ binding motif, (S/T)X(A/V) at the extreme C-terminus of PDZ ligand [1,22,23], it also sometimes bind with class II and class III PDZ binding motifs, Ψ-X- Ψ-COOH and D/E-X-Ψ-COOH (where X is any amino acid and Ψ is hydrophobic), respectively [24,25]. The GH1 domain within the N-terminal region mediates its dimerization while the GH2 of the C-terminal region is capable of interacting with retrograde protein myosin VI (MYO6) [26,27]. As a result, GIPC functions as an adaptor for loading PDZ-binding proteins as cargo for the MYO6 motor protein to traffic various transmembrane proteins into endocytic vesicles [20]. Indeed, GIPC proves necessary for proper recycling, endosomal signaling and cell surface expression of transmembrane proteins such as RTKs [28,29], GPCR [30,31], TGFβRIII [32], endoglin [33], insulin-like growth factor-1 receptor (IGF-1R) [34], LHCGR [35], and VEGFR [36,37]. GIPC also affects actin bundle stabilization, cell adhesion, cytokinesis and cell migration through interaction with proteins like α-actinin-1 [24], E-cadherin [38] and integrins [5,27]. While the majority of PDZ ligands for GIPC are transmembrane proteins, some are distinctly intracellular proteins like APPL [28] and RGS 19 [1] while others are prominent viral oncoproteins (e.g., HBc [39], E6 [21], and Tax [23]) (Figure 1). Accordingly, dysregulation of the GIPC activity, either through loss or overexpression, can disrupt crosstalk with multiple growth factor and cell survival signaling networks which ultimately help drive oncogenesis and tumor progression.

GIPC-mediated Regulation of Signaling Pathways in Cancer

Over the years there has been a steady rise in the number of GIPC targets in different cancer contexts (Figure 2 and Table 1). Here we discuss the 4

major pathways and their signaling components through which GIPC either promotes or suppresses tumorigenesis and disease progression.

GIPC-Neuropilins

Among the many binding targets of GIPC in cancer, arguably the most studied are the neuropilins (NRP1 and NRP2) [15,17,18,40,41], which are VEGF coreceptors [42–44] generally expressed in the neuronal and vascular systems but also found in a number of tumor cells [45,46]. Studies have demonstrated the GIPC-NRP association as a major driver of glioma [18], pancreatic cancer [40,41], skin cancer [15], and ECS cell (ECS) tumor [17].

While NRP1 has been previously shown to promote glioma progression through increased cellular proliferation, invasion and migration, more recent mechanistic evidence demonstrates that GIPC may positively regulate these effects through enhanced clathrin-vesicle trafficking and recycling of this receptor, which drives the oncogenic KRAS-ERK signaling pathway [18]. In ECS, GIPC binds to both NRP1/VEGFA and α6/β4-integrin via a GIPC homodimer. By forming this complex, GIPC facilitates NRP1/VEGFA/α6/β4-integrin signaling that triggers downstream PI3K/PDK1, LATS1, YAP1, and ΔNp63α-dependent ECS cell survival and tumor formation [17]. In pancreatic ductal adenocarcinoma, both GIPC and NRP1/NRP2 are frequently overexpressed and their knockdown has been shown to inhibit cell proliferation and migration while enhancing apoptosis to suppress overall tumor growth [40]. However, the underlying mechanism for the GIPC/NRP1-induced tumor promoting effects remain unresolved. Aside from these tumor-intrinsic properties, GIPC also supports the tumor microenvironment by influencing NRP1-α5β1 integrin mediated
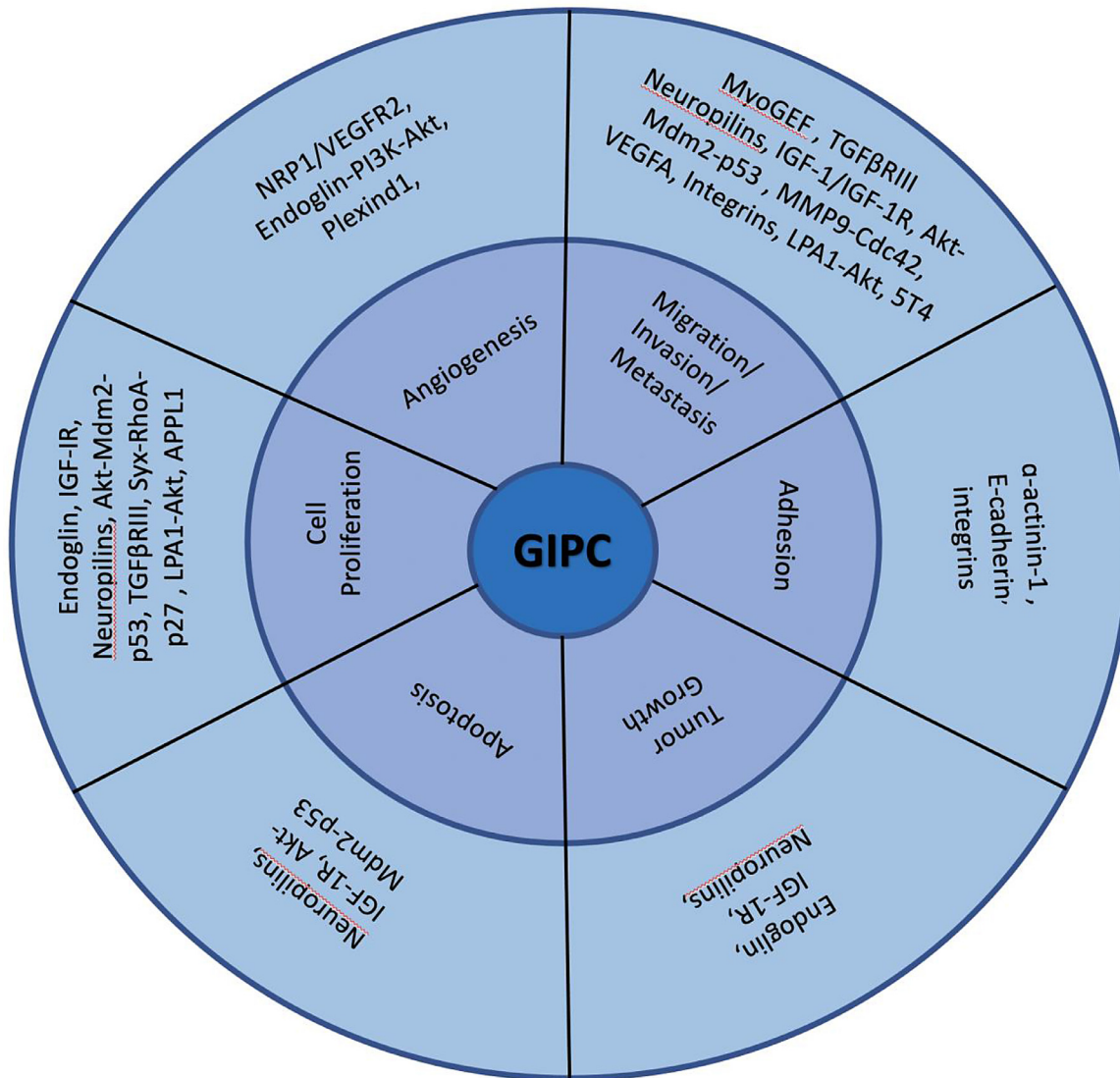
Figure 2. GIPC as a critical node for signaling networks in cancer. GIPC interacts with membrane receptors and intracellular signaling proteins to differentially regulate tumor cell biology and vascularization.

fibronectin fibril assembly in myofibroblasts. Here GIPC scaffolds NRP1 with ABL1, a tyrosine kinase that mediates NRP1 signaling for $\alpha5\beta1$ activation, leading to fibronectin fibril assembly, matrix stiffness, and tumor growth in rodent models [47].

Additionally, the GIPC-NRP interaction strongly supports the tumor microenvironment through neovascularization. Indeed, while both NRPs and GIPC are independently required to promote angiogenesis in developmental and various tumor settings [36,46,48,49], the GIPC/NRP1 interaction is also specifically necessary for efficient VEGFR2 trafficking and recycling to enhance the proangiogenic effects including endothelial migration during sprouting [37,50,51]. Indeed, this vascular function has now been implicated in the growth and metastasis of a vast majority of solid cancers [52–56]. Many preclinical models have shown that targeting NRP1 and/or VEGF exerts antitumor activity by reducing tumor angiogenesis, although the clinical benefits including overall survival in various cancers are still under evaluation [57–63]. Surprisingly, there have been no direct studies on the role of GIPC in tumor angiogenesis or its involvement in NRP1/VEGFR2 signaling during tumor vascularization.

GIPC-IGF-1R

GIPC interaction with IGF-1R is another notable association strongly implicated in the progression of prominent cancers including breast cancer [64], pancreatic cancer [12], and colorectal cancer [13]. IGF-1 signals through the IGF-1 receptor to promote growth and survival, and GIPC has been shown to positively regulate these cellular processes through IGF-1R protein stabilization by inhibiting its proteasomal degradation [11,12,34]. Studies have shown that lipopeptides (e.g., CR1023 and CR1166) which target the GIPC PDZ domain can significantly reduce tumor growth by blocking the GIPC-IGF-1R interaction and thus reducing IGF-1R expression in both breast cancer and pancreatic cancer mouse models [65]. A similar result was observed in *in vitro* studies showing decreased cell proliferation and enhanced apoptosis with reduced IGF-1R expression upon PDZ blocking peptide treatment [11].

GIPC mediates IGF/1GF-1R-induced tumor growth through reactive oxygen species (ROS) generation, which in turn, regulates phosphatases

Table 1

GIPC mediated key signaling pathways in different cancers.

Cancer Type	GIPC Mediated Signaling Pathways	Effects on Cancer Cell	References
Pancreatic Cancer	GIPC-endoglin	Increases cell proliferation and tumor growth	[82]
	GIPC-IGF-1R	Increases cell proliferation and tumor growth	[11]
	GIPC-NRP1/NRP2	Increases cell proliferation, cell survival, migration and tumor growth	[40]
Breast Cancer	GIPC-IGF-1/IGF-1R-ROS	Increases cell proliferation and migration	[64]
	GIPC/Akt-Mdm2-p53	Increases cell proliferation, cell survival and migration.	[91]
	GIPC-MMP-9-Cdc42	Increases invasion	[91]
	GIPC- TGFβRIII	Inhibits cell migration and invasion	[90]
	GIPC-MyoGEF	Increases cell polarization and invasion	[93]
Colorectal Cancer	GIPC-IGF-1-ROS-ZNF143	ROS generation and tumorigenesis	[13]
Skin Cancer	GIPC-Syx-RhoA-p27	Increases cell proliferation	[15]
ECS Tumor	GIPC-VEGF-A/NRP1/α6/β4-integrin; LATS1/YAP1/∆Np63α	Increases cell invasion, migration, cell survival and tumor formation	[17]
Glioma Tumors	GIPC/NRP1-APPL1, p130Cas, KRAS-ERK.	Increases cell proliferation, invasion and cell survival	[18]
Cervical cancer (Associated with HPV-18 infection)	GIPC-E6 of HPV 18	GIPC degradation increases cell proliferation	[21]
Melanoma	GIPC-APPL-TRP1	Promotes melanogenesis in melanocytes	[16]

involved in various cancer cell proliferation [64]. In colon cancer, GIPC is believed to play a role in tumorigenesis and survival by inducing zinc finger protein, ZNF143 expression as part of the IGF-1/IGF-1R-ROS cascade [13]. Interestingly, in these cancer cell models, the role of GIPC in IGF/IGF-1R-induced cell proliferation proved to be independent of the canonical MAPK/ERK or PI3K/Akt pathways [13,64]. Therefore, the crucial downstream mechanisms by which GIPC drives ROS generation and tumor cell proliferation through the IGF/IGF-1R signaling system remain to be fully elucidated.

GIPC-LPA1/AKT Pathways

Aside from its above-mentioned roles as a tumor promoter, GIPC can also function as a powerful tumor suppressor often in the same cancer types depending on its ligands. Lysophosphatidic acid 1 (LPA1) is a receptor for LPA that has been shown to promote various carcinomas including breast, prostate, colon, and pancreatic cancer by regulating cell motility, chemotaxis, migration, and proliferation [66–69]. Here, GIPC can oppose these functions by directly binding to the PDZ binding motif of LPA1 to form an endocytic complex with APPL, which promotes LPA1 trafficking to early endosome EEA1 [70]. This in turn results in the downregulation of LPA1-induced Akt signaling from APPL endosome and thus inhibits the cell proliferation and cell migration [70]. Accordingly, GIPC depletion has been shown to delay LPA1 trafficking to EEA1 endosomes and thus maintains the LPA1/APPL association in signaling endosomes to promote Akt signaling towards higher cell proliferation and cell motility [70]. Consistent with the above findings upon GIPC depletion, recent evidence further suggests that disrupting this protein interaction with a point mutation in the LPA1 PDZ-binding motif induces oncogenic transformation by elevating Akt mediated cell proliferation [71].

Similar to LPA1, GIPC depletion has been reported to delay TrkA trafficking from APPL signaling endosome to early endosome [28], although their functional outcomes can differ significantly. The delay in TrkA trafficking has been shown to reduce Akt and Erk signaling [28], whereas the delay in LPA1 trafficking enhances the Akt signaling for cellular proliferation [70]. This indicates that the GIPC-APPL/Akt signaling pathway can yield highly divergent effects depending on its target proteins even within the

same cancer cell type, suggesting that the overall strength and duration of the interaction between GIPC and its ligands serve as fundamentally crucial yet elusive determinants of cancer progression. Further highlighting the complexity of these GIPC-dependent signaling networks, GIPC has also been reported to bind directly to APPL to form a GIPC-APPL/Akt complex [16]. Hence, precisely how GIPC preferentially interacts with these trafficking components to modulate the oncogenic properties of Akt signaling remains to be fully elucidated.

GIPC Regulation of the TGF-beta pathways

GIPC-Endoglin

Endoglin is a specialized TGFβ co-receptor expressed in proliferating endothelial cells of normal and tumor vessels [72–74]. GIPC binds to this coreceptor through the C-terminal PDZ-binding motif SMA, which promotes endoglin cell surface retention and potentiates canonical Smad1/5/8 signaling downstream to promote angiogenesis [33]. While endoglin has received considerable attention as a vascular target in many solid cancers [73,75–77], more recent findings in cell lines and patient samples demonstrate that endoglin is also expressed in a subset of human breast [78], pancreatic [79], colorectal [80], and prostate cancer cells [81], which raises the question of the precise role of GIPC in regulating the tumor-intrinsic versus vascular endoglin in the surrounding tumor vessels. Notably, in pancreatic cancer, Pal et al., has shown that the GIPC-endoglin interaction is necessary for cell proliferation as blocking this interaction via a peptide-based inhibitor AP1032 inhibited *in vivo* tumor growth, and induced differentiation while also sensitizing pancreatic cancer cells to the frontline chemotherapeutic drug gemcitabine [82]. Furthermore, similar to the vascular system, inhibiting the GIPC-endoglin interaction also abrogated Smad 1/5/8 activation in pancreatic tumor cells [82].

Interestingly, the enhanced Smad 1/5/8 activation mediated by the GIPC-endoglin complex serves to inhibit endothelial cell migration [33], whereas GIPC stimulates migration when it interacts with NRP1 [51]. This dynamic again exemplifies the versatility of GIPC in differentially regulating cellular functions depending on the PDZ ligand, although precisely how GIPC favors one PDZ ligand over another, such as the case in endoglin versus NRP1,

remains to be characterized. In any event, it is also notable that the GIPC-endoglin interaction also regulates noncanonical signaling functions at least in the vascular system. In one study, GIPC has been shown to scaffold PI3K to endoglin at the plasma membrane to promote PI3K/Akt activation which acts to stabilize endothelial capillary sprouts during developmental angiogenesis [83]. While it is unclear whether GIPC functions in a similar manner towards endoglin signaling in pancreatic cancer cells, targeting the endoglin/GIPC complex may be an effective dual strategy to counter the tumorigenic and angiogenic properties of endoglin in certain tumor types.

GIPC-TGFβRIII

TGFβRIII is another coreceptor of the transforming growth factor-β (TGF-β) superfamily that shares structural homology with endoglin but is much more widely expressed [84]. Previous studies have established numerous roles for this receptor protein including tumor suppressive effects in certain cancers in part by inhibiting tumor motility and invasion *in vitro* [85–89] and angiogenesis, invasion and metastasis *in vivo* [86]. As was observed for the GIPC-endoglin interaction in endothelial cells, GIPC also directly interacts with TGFβRIII through the PDZ-binding motif to facilitate TGFβRIII cell surface stability, expression and TGFβ responsiveness for Smad2/3 activation [32], which potentiates the suppressive effects of TGFβRIII on cell proliferation, migration and invasion in breast cancer [90].

Loss of GIPC expression, as evidenced in HPV-18-associated cervical cancer, appears to dampen the tumor suppressive effects of certain PDZ ligands including TGFβRIII. Studies have shown that GIPC interacts with E6 oncoprotein of HPV-18 which induces GIPC polyubiquitination for proteasomal degradation [21]. As GIPC enhances TGFβRIII protein stability and responsiveness to TGFβ, E6-mediated GIPC degradation results in higher proliferation due to decreased antiproliferative effects of TGFβ signaling [21]. This mechanism may apply similarly to many other cancers including primary kidney tumors, primary prostate cancer and primary colorectal tumor where GIPC gene expression is downregulated [20] and therefore GIPC may exhibit as a tumor suppressor role in such cancers.

Targeting GIPC in cancer

As GIPC is highly expressed in a number of human malignancies, inhibiting its activity can be an effective strategy for cancer therapy. Indeed, multiple studies have already demonstrated *in vivo* and *in vitro* antitumor activity by targeting GIPC, both through RNAi-mediated knockdown and by peptide-based competitive inhibition of GIPC-PDZ specific interactions [11,14,15,40,82,91,92]. GIPC depletion was shown to promote apoptosis [14,40], G2 cell-cycle arrest [14,40], and autophagy [92] while impairing cell proliferation [14,40,91], motility [14], invasion [40,91,93] and tumor growth [11,40,91], in breast cancer [14,91,93], colorectal cancer [14], and pancreatic cancer [11,40,92]. In some cases, GIPC silencing [92] and inhibition of the GIPC-endoglin interaction [82] also influences therapeutic efficacy as evidenced by the sensitization of pancreatic cancer cell lines to chemotherapeutic drug gemcitabine. In particular, data shows that GIPC depletion promotes this effect by causing exosome exocytosis of drug resistance gene ATP-binding cassette sub-family G member 2 (ABCG2), rendering it nonfunctional and thus sensitizing the cells to gemcitabine [92]. In another study, Patra et al., developed a series of cell-permeable lipopeptides that selectively block the PDZ domain of GIPC to elicit inhibitory effects against pancreatic and breast cancers in both cellular and animal models [65]. By mimicking the unique C-terminal PDZ-binding motif of GAIP, especially the prevalent SEA motif, these lipopeptides appear to competitively block the protein-protein interactions occurring between GIPC and PDZ ligand proteins [65]. Among the peptides, CR1166 was found most efficient in binding to the GIPC PDZ domain to disrupt the GIPC/IGF-1R association,

which consequently inhibits IGF-1R activity, cell proliferation and tumor growth [65]. Likewise, in skin cancer competitive inhibition of the GIPC/Syx interaction with a PDZ blocking peptide prevented RhoA activation which potently inhibited cell proliferation [15].

RNAi-mediated knockdown is another potential approach of targeting GIPC function. Although siRNA-mediated drugs have their own caveats like poor stability and tissue penetration [94], polymeric nanoparticle mediated siRNA delivery is considered a remedy to overcome the aforementioned obstacles [95–97]. As demonstrated by Borchardt et al., nanoparticle based GIPC-siRNA treatment can significantly reduce tumor volume in animal models with minimal adverse effects [40].

However, like the vast majority of targeted therapies in human clinical trials, both peptide-based and siRNA approaches must fully consider the unanticipated off-target effects of abrogating the essential homeostatic actions of GIPC. Conversely, because GIPC can act as a tumor suppressor and is downregulated in some cancers, alternative approaches should include identifying and targeting the negative regulators of GIPC. Taken together, these findings suggest that GIPC can serve as a promising therapeutic target in various human cancers but its expression pattern and dominant functional roles in each tumor type must be carefully considered.

Conclusion

GIPC is a versatile adaptor protein that regulates the functional trafficking of RTKs, GPCRs, TGFβ receptors, and integrins. The dynamic actions of GIPC is highlighted by its vastly diverse signaling network of molecular partners, their interplay and biological consequences. Increasing evidence of its dichotomous roles in cancer clearly warrants further investigation into not only more selective GIPC-targeted therapies but also understanding the quantitative aspects of the strength and duration with which these ligands bind to GIPC. A clear grasp of these molecular mechanisms will be crucial to deciphering how these dynamic processes are altered in various cancers.

References

[1] De Vries L, Lou X, Zhao G, Zheng B, Farquhar MG. GIPC, a pdz domain containing protein, interacts specifically with the C terminus of RGS-GAIP. *Proc Natl Acad Sci U S A* 1998;**95**(21):12340–5. doi:10.1073/pnas.95.21.12340.

[2] Wu J, O'Donnell M, Gitler AD, Klein PSK. 2/XGIPC, an IGF1 receptor interacting protein, is required for IGF signaling in Xenopus Eye development. *Development* 2006;**133**(18):3651–60. doi:10.1242/dev.02547.

[3] Ponting CP, Phillips C, Davies KE, Blake DJPDZD. Targeting signalling molecules to sub-membranous sites. BioEssays *John Wiley and Sons Inc* 1997:469–79. doi:10.1002/bies.950190606.

[4] Fanning AS, Anderson JM. Protein-protein interactions: PDZ domain networks. *Curr Biol* 1996;**6**(11):1385–8. doi:10.1016/S0960-9822(96)00737-3.

[5] Spicer E, Suckert C, Al-Attar H, Marsden M. Integrin A5β1 function is regulated by XGIPC/Kermit2 mediated endocytosis during Xenopus Laevis gastrulation. *PLoS One* 2010;5(5):5. doi:10.1371/journal.pone.0010665.

[6] Gao Y, Li M, Chen W, Simons MS. Syndecan-4 cytoplasmic domain binding PDZ protein, inhibits cell migration. *J Cell Physiol* 2000;**184**(3):373–9. https://doi.org/10.1002/1097-4652(200009)184:3⟨373::AID-JCP12⟩3.0.CO;2-I.

[7] Katoh M. Functional proteomics, human genetics and cancer biology of GIPC family members. *Exp Mol Med* 2013;**45**(6):e26–9. doi:10.1038/emm.2013.49.

[8] Salama O, Herrmann S, Tziknovsky A, Piura B, Meirovich M, Trakht I, Reed B, Lobel LI, Marks RS. Chemiluminescent optical fiber immunosensor for detection of autoantibodies to ovarian and breast cancer-associated antigens. *Biosens Bioelectron* 2007;**22**(7):1508–16. doi:10.1016/j.bios.2006.07.003.

[9] Amir T, Kohn H, Delgado B, Rabinovich A, Piura B, Chan G, Kalantarov G, Yavelsky V, Rohkin S, Shaco-Levy R, et al. Native Human autoantibodies targeting GIPC1 identify differential expression in malignant tumors of the breast and ovary. *BMC Cancer* 2008;**8**:1–11. doi:10.1186/1471-2407-8-247.

[10] Rudchenko S, Scanlan M, Kalantarov G, Yavelsky V, Levy C, Estabrook A, Old L, Chan GL, Lobel L, Trakht I. A human monoclonal autoantibody to breast cancer identifies the PDZ domain containing protein GIPC1 as a novel breast cancer-associated antigen. *BMC Cancer* 2008;**8**:1–8. doi:10.1186/1471-2407-8-248.

[11] Muders MH, Vohra PK, Dutta SK, Wang E, Ikeda Y, Wang L, Udugamasooriya DG, Memic A, Rupashinghe CN, Baretton GB, et al. Targeting GIPC/synectin in pancreatic cancer inhibits tumor growth. *Clin Cancer Res* 2009;**15**(12):4095–103. doi:10.1158/1078-0432.CCR-08-2837.

[12] Muders MH, Dutta SK, Wang L, Lau JS, Bhattacharya R, Smyrk TC, Chari ST, Datta K, Mukhopadhyay D. Expression and regulatory role of GAIP-interacting protein GIPC in pancreatic adenocarcinoma. *Cancer Res* 2006;**66**(21):10264–8. doi:10.1158/0008-5472.CAN-06-2321.

[13] Paek AR, You HJ. GAIP-interacting protein, C-terminus is involved in the induction of zinc-finger protein 143 in response to insulin-like growth factor-1 in colon cancer cells. *Mol Cells* 2011;**32**(5):415–19. doi:10.1007/s10059-011-0078-7.

[14] Chittenden TW, Pak J, Rubio R, Cheng H, Holton K, Prendergast N, Glinskii V, Cai Y, Culhane A, Bentink S, et al. Therapeutic implications of GIPC1 silencing in cancer. *PLoS One* 2010(12):5. doi:10.1371/journal.pone.0015581.

[15] Yoshida A, Shimizu A, Asano H, Kadonosono T, Kondoh SK, Geretti E, Mammoto A, Klagsbrun M, Seo MK. VEGF-A/NRP1 stimulates GIPC1 and Syx complex formation to promote RhoA activation and proliferation in skin cancer cells. *Biol Open* 2015;**4**(9):1063–76. doi:10.1242/bio.010918.

[16] Kedlaya R, Kandala G, Liu TF, Maddodi N, Devi S, Setaluri V. Interactions between GIPC-APPL and GIPC-TRP1 regulate melanosomal protein trafficking and melanogenesis in human melanocytes. *Arch Biochem Biophys* 2011;**508**(2):227–33. doi:10.1016/j.abb.2011.01.021.

[17] Grun D, Adhikary G, Eckert RL. NRP-1 interacts with GIPC1 and A6/B4-integrins to increase YAP1/ΔNp63α-dependent epidermal cancer stem cell survival. *Oncogene* 2018;**37**(34):4711–22. doi:10.1038/s41388-018-0290-4.

[18] Zhang G, Chen L, Sun K, Khan AA, Yan J, Liu H, Lu A, Gu N. Neuropilin-1 (NRP-1)/GIPC1 pathway mediates glioma progression. *Tumor Biol* 2016;**37**(10):13777–88. doi:10.1007/s13277-016-5138-3.

[19] Mikhaylenko DS, Lyubchenko LN, Zborovskaya IB, Strelnikov VV, Zaletayev DV. Analysis of polymorphic variants of gene GIPC1 CGG repeats in healthy individuals and in patients with breast cancer and non-small cell lung cancer. *Genetika* 2005;**41**(9):1289–93.

[20] Kirikoshi H, Katoh M. Expression of human GIPC1 in normal tissues, cancer cell lines, and primary tumors. *Int J Mol Med* 2002;**9**(5):509–13. doi:10.3892/ijmm.9.5.509.

[21] Favre-Bonvin A, Reynaud C, Kretz-Remy C, Jalinot P. Human papillomavirus type 18 E6 protein binds the cellular PDZ protein TIP-2/GIPC, which is involved in transforming growth factor β signaling and triggers its degradation by the proteasome. *J Virol* 2005;**79**(7):4229–37. doi:10.1128/jvi.79.7.4229-4237.2005.

[22] Wang L, Kalb RG, Strittmatter SM, Vries D, Natl MGP, Sci A. A PDZ protein regulates the distribution of the transmembrane semaphorin. *M-SemF* * 1999;**274**(20):14137–46.

[23] Rousset R, Fabre S, Desbois C, Bantignies F, Jalinot P. The C-terminus of the HTLV-1 tax oncoprotein mediates interaction with the PDZ domain of cellular proteins. *Oncogene* 1998;**16**(5):643–54. doi:10.1038/sj.onc.1201567.

[24] Bunn RC, Jensen MA, Reed BC. Protein interactions with the glucose transporter binding protein GLUT1CBP that provide a link between GLUT1 and the cytoskeleton. *Mol Biol Cell* 1999;**10**(4):819–32. doi:10.1091/mbc.10.4.819.

[25] Nourry, C, Grant, SGN, Borg, J R EVIEW PDZ domain proteins : plug and play ! 2003, No. April, 1–13.

[26] Arden SD, Puri C, Au JSY, Kendrick-Jones J, Buss F. Myosin VI is required for targeted membrane transport during cytokinesis. *Mol Biol Cell* 2007;**18**(12):4750–61. doi:10.1091/mbc.E07-02-0127.

[27] Naccache SN, Hasson T, Horowitz A. Binding of internalized receptors to the PDZ domain of GIPC/synectin recruits myosin VI to endocytic vesicles. *Proc Natl Acad Sci U S A* 2006;**103**(34):12735–40. doi:10.1073/pnas.0605317103.

[28] Varsano T, Dong M-Q, Niesman I, Gacula H, Lou X, Ma T, Testa JR, Yates JR, Farquhar MG. GIPC is recruited by APPL to peripheral TrkA endosomes and regulates TrkA trafficking and signaling. *Mol Cell Biol* 2006;**26**(23):8942–52. doi:10.1128/mcb.00305-06.

[29] Lin DC, Quevedo C, Brewer NE, Bell A, Testa JR, Grimes ML, Miller FD, Kaplan DR. APPL1 associates with TrkA and GIPC1 and is required for nerve growth factor-mediated signal transduction. *Mol Cell Biol* 2006;**26**(23):8928–41. doi:10.1128/mcb.00228-06.

[30] Hu LA, Chen W, Martin NP, Whalen EJ, Premont RT, Lefkowitz RJ. GIPC interacts with the B1-adrenergic receptor and regulates B1-adrenergic receptor-mediated ERK activation. *J Biol Chem* 2003;**278**(28):26295–301. doi:10.1074/jbc.M212352200.

[31] Jeanneteau F, Guillin O, Diaz J, Griffon N, Sokoloff P. GIPC recruits GAIP (RGS19) to attenuate dopamine D2 receptor signaling. *Mol Biol Cell* 2004;**15**(11):4926–37. doi:10.1091/mbc.E04-04-0285.

[32] Blobe GC, Liu X, Fang SJ, How T, Lodish HF. A novel mechanism for regulating transforming growth factor β (TGF-β) signaling: functional modulation of type III TGF-β receptor expression through interaction with the PDZ domain protein, GIPC. *J Biol Chem* 2001;**276**(43):39608–17. doi:10.1074/jbc.M106831200.

[33] Lee NY, Ray B, How T, Blobe GC. Endoglin promotes transforming growth factor β-mediated Smad 1/5/8 signaling and inhibits endothelial cell migration through its association with GIPC. *J Biol Chem* 2008;**283**(47):32527–33. doi:10.1074/jbc.M803059200.

[34] Booth RA, Cummings C, Tiberi M, Johné Liu X. GIPC participates in G protein signaling downstream of insulin-like growth factor 1 receptor. *J Biol Chem* 2002;**277**(8):6719–25. doi:10.1074/jbc.M108033200.

[35] Hirakawa T, Galet C, Kishi M, Ascoli M. GIPC binds to the human lutropin receptor (HLHR) through an unusual PDZ domain binding motif, and it regulates the sorting of the internalized human choriogonadotropin and the density of cell surface HLHR. *J Biol Chem* 2003;**278**(49):49348–57. doi:10.1074/jbc.M306557200.

[36] Chittenden TW, Claes F, Lanahan AA, Autiero M, Palac RT, Tkachenko EV, Elfenbein A, Ruiz de Almodovar C, Dedkov E, Tomanek R, et al. Selective regulation of arterial branching morphogenesis by synectin. *Dev Cell* 2006;**10**(6):783–95. doi:10.1016/j.devcel.2006.03.012.

[37] Prahst C, Héroult M, Lanahan AA, Uziel N, Kessler O, Shraga-Heled N, Simons M, Neufeld G, Augustin HG. Neuropilin-1-VEGFR-2 complexing requires the PDZ-binding domain of Neuropilin-1. *J Biol Chem* 2008;**283**(37):25110–14. doi:10.1074/jbc.C800137200.

[38] Knudsen KA, Soler AP, Johnson KR, Wheelock MJ. Interaction of α-Actinin with the cadherin/catenin cell-cell adhesion complex via α-Catenin. *J Cell Biol* 1995;**130**(1):67–77. doi:10.1083/jcb.130.1.67.

[39] Razanskas R, Sasnauskas K. Interaction of Hepatitis B virus core protein with human GIPC1. *Arch Virol* 2010;**155**(2):247–50. doi:10.1007/s00705-009-0561-z.

[40] Borchardt H, Schulz A, Datta K, Muders MH, Aigner A. Silencing of Neuropilins and GIPC1 in pancreatic ductal adenocarcinoma exerts multiple cellular and molecular antitumor effects. *Sci Rep* 2019;**9**(1):1–12. doi:10.1038/s41598-019-51881-8.

[41] Muders H. M. Neuropilin and Neuropilin associated molecules as new molecular targets in pancreatic adenocarcinoma. *Anticancer. Agents Med Chem.* 2012;**11**(5):442–7. doi:10.2174/187152011795677481.

[42] Makinen T, Olofsson B, Karpanen T, Hellman U, Soker S, Klagsbrun M, Eriksson U, Alitalo K. Differential binding of vascular endothelial growth factor B splice and proteolytic isoforms to Neuropilin-1. *J Biol Chem* 1999;**274**(30):21217–22. doi:10.1074/jbc.274.30.21217.

[43] Wise LM, Veikkola T, Mercer AA, Savory LJ, Fleming SB, Caesar C, Vitali A, Makinen T, Alitalo K, Stacker SA. Vascular endothelial growth factor (VEGF)-like protein from Orf virus NZ2 binds to VEGFR2 and Neuropilin-1. *Proc Natl Acad Sci U S A* 1999;**96**(6):3071–6. doi:10.1073/pnas.96.6.3071.

[44] Gluzman-Poltorak Z, Cohen T, Herzog Y, Neufeld G. Neuropilin-2 and Neuropilin-1 are receptors for the 165-amino acid form of vascular endothelial growth factor (VEGF) and of placenta growth factor-2, but only Neuropilin-2 functions as a receptor for the 145-amino acid form of VEGF. *J Biol Chem* 2000;**275**(24):18040–5. doi:10.1074/jbc.M909259199.

[45] Soker S, Takashima S, Miao HQ, Neufeld G, Klagsbrun M. Neuropilin-1 is expressed by endothelial and tumor cells as an isoform- specific receptor for vascular endothelial growth factor. *Cell* 1998;**92**(6):735–45. doi:10.1016/S0092-8674(00)81402-6.

[46] Miao H, Lee P, Lin H, Soker S, Klagsbrun M. Neuropilin-1 expression by tumor cells promotes tumor angiogenesis and progression. *FASEB J* 2000;**14**(15):2532–9. doi:10.1096/fj.00-0250com.

[47] Yaqoob U, Cao S, Shergill U, Jagavelu K, Geng Z, Yin M, De Assuncao TM, Cao Y, Szabolcs A, Thorgeirsson S, et al. Neuropilin-1 stimulates tumor growth by increasing fibronectin fibril assembly in the tumor microenvironment. *Cancer Res* 2012;**72**(16):4047–59. doi:10.1158/0008-5472.CAN-11-3907.

[48] Miao HQ, Klagsbrun M. Neuropilin is a mediator of angiogenesis. *Cancer Metastasis Rev Springer* 2000:29–37. doi:10.1023/A:1026579711033.

[49] Kawasaki T. *Vascular Anomaly in Neuropilin-1 Mutant Mice*; 1999.

[50] Lanahan AA, Hermans K, Claes F, Kerley-Hamilton JS, Zhuang ZW, Giordano FJ, Carmeliet P, Simons M. VEGF receptor 2 endocytic trafficking regulates arterial morphogenesis. *Dev Cell* 2010;**18**(5):713–24. doi:10.1016/j.devcel.2010.02.016.

[51] Wang L, Mukhopadhyay D, Xu X, Wang L, Mukhopadhyay D, Xu X. C terminus of RGS-GAIP-interacting protein conveys Neuropilin-1-mediated signaling during angiogenesis. *FASEB J* 2006;**20**(9):1513–15. doi:10.1096/fj.05-5504fje.

[52] Prud'homme GJ, Glinka Y. Neuropilins are multifunctional coreceptors involved in tumor initiation, growth, metastasis and immunity. *Oncotarget.* 2012:921–39 Impact Journals LLC September 3,. doi:10.18632/oncotarget.626.

[53] Latil A, Bièche I, Pesche S, Valéri A, Fournier G, Cussenot O, Lidereau R. VEGF overexpression in clinically localized prostate tumors and neuropilin-1 overexpression in metastatic forms. *Int J Cancer* 2000;**86**(3):167–71. https://doi.org/10.1002/(sici)1097-0215(20000320)89:2⟨167::aid-ijc11⟩3.0.co 2-9.

[54] Lei X, Duda DG, Di Tomaso E, Ancukiewicz M, Chung DC, Lauwers GY, Samuel R, Shellito P, Czito BG, Lin PC, et al. Direct evidence that bevacizumab, an anti-VEGF antibody, up-regulates SDF1α, CXCR4, CXCL6, and Neuropilin 1 in tumors from patients with rectal cancer. *Cancer Res* 2009;**69**(20):7905–10. doi:10.1158/0008-5472.CAN-09-2099.

[55] Ghosh S, Sullivan CAW, Zerkowski MP, Molinaro AM, Rimm DL, Camp RL, Chung GG. High levels of vascular endothelial growth factor and its receptors (VEGFR-1, VEGFR-2, Neuropilin-1) are associated with worse outcome in breast cancer. *Hum Pathol* 2008;**39**(12):1835–43. doi:10.1016/j.humpath.2008.06.004.

[56] Morin E, Sjöberg E, Tjomsland V, Testini C, Lindskog C, Franklin O, Sund M, Öhlund D, Kiflemariam S, Sjöblom T, et al. VEGF receptor-2/Neuropilin 1 trans-complex formation between endothelial and tumor cells is an independent predictor of pancreatic cancer survival. *J Pathol* 2018;**246**(3):311–22. doi:10.1002/path.5141.

[57] Starzec A, Vassy R, Martin A, Lecouvey M, Di Benedetto M, Crépin M, Perret GY. Antiangiogenic and antitumor activities of peptide inhibiting the vascular endothelial growth factor binding to Neuropilin-1. *Life Sci* 2006;**79**(25):2370–81. doi:10.1016/j.lfs.2006.08.005.

[58] Pan Q, Chanthery Y, Liang WC, Stawicki S, Mak J, Rathore N, Tong RK, Kowalski J, Yee SF, Pacheco G, et al. Blocking Neuropilin-1 function has an additive effect with anti-VEGF to inhibit tumor growth. *Cancer Cell* 2007;**11**(1):53–67. doi:10.1016/j.ccr.2006.10.018.

[59] Hong TM, Chen YL, Wu YY, Yuan A, Chao YC, Chung YC, Wu MH, Yang SC, Pan SH, Shih JY, et al. Targeting Neuropilin 1 as an antitumor strategy in lung cancer. *Clin Cancer Res* 2007;**13**(16):4759–68. doi:10.1158/1078-0432.CCR-07-0001.

[60] Zeng F, Luo F, Lv S, Zhang H, Cao C, Chen X, Wang S, Li Z, Wang X, Dou X, et al. A monoclonal antibody targeting neuropilin-1 inhibits adhesion of MCF7 breast cancer cells to fibronectin by suppressing the FAK/P130cas signaling pathway. *Anticancer Drugs* 2014;**25**(6):663–72. doi:10.1097/CAD.0000000000000091.

[61] Graziani G, Lacal PM. Neuropilin-1 as therapeutic target for malignant melanoma. *Front Oncol* 2015:125 Frontiers Media S.A. June 3,. doi:10.3389/fonc.2015.00125.

[62] Weekes CD, Beeram M, Tolcher AW, Papadopoulos KP, Gore L, Hegde P, Xin Y, Yu R, Shih LM, Xiang H, et al. A phase I study of the human monoclonal anti-NRP1 antibody MNRP1685A in patients with advanced solid tumors. *Invest New Drugs* 2014;**32**(4):653–60. doi:10.1007/s10637-014-0071-z.

[63] Xin Y, Li J, Wu J, Kinard R, Weekes CD, Patnaik A, LoRusso P, Brachmann R, Tong RK, Yan Y, et al. Pharmacokinetic and pharmacodynamic analysis of circulating biomarkers of anti-NRP1, a novel antiangiogenesis agent, in two phase i trials in patients with advanced solid tumors. *Clin Cancer Res* 2012;**18**(21):6040–8. doi:10.1158/1078-0432.CCR-12-1652.

[64] Seung J, Paek AR, Youl S, Jin H. GIPC mediates the generation of reactive oxygen species and the regulation of cancer cell proliferation by insulin-like growth factor-1/IGF-1R signaling. *Cancer Lett* 2010;**294**(2):254–63. doi:10.1016/j.canlet.2010.02.007.

[65] Patra CR, Rupasinghe CN, Dutta SK, Bhattacharya S. *Chemically Modified Peptides Targeting the PDZ Domain of GIPC as a Therapeutic Approach for Cancer*; 2012.

[66] Jonkers J, Moolenaar WH. Mammary tumorigenesis through LPA receptor signaling. *Nat Struct Mol Biol* 2007;**26**:301–34. doi:10.1016/j.ccr.2009.05.003.

[67] Guo R, Kasbohm EA, Arora P, Sample CJ, Baban B, Sud N, Sivashanmugam P, Moniri NH, Daaka Y. Expression and function of lysophosphatidic acid LPA1 receptor in prostate cancer cells. *Endocrinology* 2006;**147**(10):4883–92. doi:10.1210/en.2005-1635.

[68] Shida, D, Kitayama, J, Yamaguchi, H, Okaji, Y, Tsuno, NH, Watanabe, T, Takuwa, Y, Nagawa, H Lysophosphatidic acid (LPA) enhances the metastatic potential of human colon carcinoma DLD1 cells through LPA1 1, *Cancer Res.* 2003, Vol. 63.

[69] Yamada T, Sato K, Komachi M, Malchinkhuu E, Tobo M, Kimura T, Kuwabara A, Yanagita Y, Ikeya T, Tanahashi Y, et al. Lysophosphatidic acid (LPA) in malignant ascites stimulates motility of human pancreatic cancer cells through LPA1. *J Biol Chem* 2004;**279**(8):6595–605. doi:10.1074/jbc.M308133200.

[70] Varsano T, Taupin V, Guo L, Baterina OY, Farquhar MG. The PDZ protein GIPC regulates trafficking of the LPA1 receptor from APPL signaling endosomes and attenuates the cell's response to LPA. *PLoS One* 2012;**7**(11). doi:10.1371/journal.pone.0049227.

[71] Shano S, Hatanaka K, Ninose S, Moriyama R, Tsujiuchi T, Fukushima N. A lysophosphatidic acid receptor lacking the PDZ-binding domain is constitutively active and stimulates cell proliferation. *Biochim Biophys Acta - Mol Cell Res* 2008;**1783**(5):748–59. doi:10.1016/j.bbamcr.2007.11.013.

[72] Miller DW, Graulich W, Karges B, Stahl S, Ernst M, Ramaswamy A, Sedlacek HH, Müller R, Adamkiewicz J. Elevated expression of endoglin, a component of the TGF-β-receptor complex, correlates with proliferation of tumor endothelial cells. *Int J Cancer* 1999;**81**(4):568–72. https://doi.org/10.1002/(SICI)1097-0215(19990517)81:4⟨568::AID-IJC11⟩3.0.CO 2-X.

[73] K Seon B, Haba A, Matsuno F, Takahashi N, Tsujie M, She X, Harada N, Uneda S, Tsujie T, Toi H, et al. Endoglin-targeted cancer therapy. *Curr Drug Deliv* 2010;**8**(1):135–43. doi:10.2174/156720111793663570.

[74] Burrows FJ, Derbyshire EJ, Tazzari PL, Amlot P, Gazdar AF, King SW, Letarte M, Vitetta ES, Thorpe PE. Up-regulation of endoglin on vascular endothelial cells in human solid tumors: implications for diagnosis and therapy. *Clin Cancer Res* 1995;**1**(12).

[75] Mendelson DS, Gordon MS, Rosen LS, Hurwitz H, Wong MK, Adams BJ, Alvarez D, Seon BK, Theuer CP, Leigh BR. Phase I study of TRC105 (anti-CD105 [Endoglin] antibody) therapy in patients with advanced refractory cancer. *J Clin Oncol* 2010;**28**(15_suppl) 3013-3013. doi:10.1200/jco.2010.28.15_suppl.3013.

[76] Uneda S, Toi H, Tsujie T, Tsujie M, Harada N, Tsai H, Seon BK. Anti-endoglin monoclonal antibodies are effective for suppressing metastasis and the primary tumors by targeting tumor vasculature HHS public access. *Int J Cancer* 2009;**125**(6):1446–53. doi:10.1002/ijc.24482.

[77] Tsujie M, Uneda S, Tsai H, Seon BK. Effective anti-angiogenic therapy of established tumors in mice by naked anti-human Endoglin (CD105) antibody: differences in growth rate and therapeutic response between tumors growing at different Sites. *Int J Oncol* 2006;**29**(5):1087–94. doi:10.3892/ijo.29.5.1087.

[78] Gómez-Esquer F, Agudo D, Martínez-Arribas F, Nuñez-Villar MJ, Schneider J. MRNA expression of the angiogenesis markers VEGF and CD105 (Endoglin) in human breast cancer. *Anticancer Res* 2004;**24**(3 A):1581–5.

[79] Yoshitomi H, Kobayashi S, Ohtsuka M, Kimura F, Shimizu H, Yoshidome H, Miyazaki M. Specific expression of endoglin (CD105) in endothelial cells of intratumoral blood and lymphatic vessels in pancreatic cancer. *Pancreas* 2008;**37**(3):275–81. doi:10.1097/mpa.0b013e3181690b97.

[80] El-Gohary YM, Silverman JF, Olson PR, Liu YL, Cohen JK, Miller R, Saad RS. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in prostatic adenocarcinoma. *Am J Clin Pathol* 2007;**127**(4):572–9. doi:10.1309/X6NXYE57DLUE2NQ8.

[81] Saad RS, Liu YL, Nathan G, Celebrezze J, Medich D, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in colorectal cancer. *Mod Pathol* 2004;**17**(2):197–203. doi:10.1038/modpathol.3800034.

[82] Pal K, Pletnev AA, Dutta SK, Wang E, Zhao R, Baral A, Yadav VK, Aggarwal S, Krishnaswamy S, Alkharfy KM, et al. Inhibition of endoglin-GIPC interaction inhibits pancreatic cancer cell growth. *Mol Cancer Ther* 2014;**13**(10):2264–75. doi:10.1158/1535-7163.MCT-14-0291.

[83] Lee NY, Golzio C, Gatza CE, Sharma A, Katsanis N, Blobe GC. Endoglin regulates PI3-kinase/Akt trafficking and signaling to alter endothelial capillary stability during angiogenesis. *Mol Biol Cell* 2012;**23**(13):2412–23. doi:10.1091/mbc.E11-12-0993.

[84] Wang XF, Lin HY, Ng-Eaton E, Downward J, Lodish HF, Weinberg RA. Expression cloning and characterization of the TGF-β Type III receptor. *Cell* 1991;**67**(4):797–805. doi:10.1016/0092-8674(91)90074-9.

[85] Mythreye K, Blobe GC. The type III TGF-β receptor regulates epithelial and cancer cell migration through β-Arrestin2-mediated activation of Cdc42. *Proc Natl Acad Sci U S A* 2009;**106**(20):8221–6. doi:10.1073/pnas.0812879106.

[86] Dong M, How T, Kirkbride KC, Gordon KJ, Lee JD, Hempel N, Kelly P, Moeller BJ, Marks JR, Blobe GC. The type III TGF-β receptor suppresses breast cancer progression. *J Clin Invest* 2007:117. doi:10.1172/JCI29293.

[87] Gordon KJ, Dong M, Chislock EM, Fields TA, Blobe GC. Loss of type III transforming growth factor β receptor expression increases motility and invasiveness associated with epithelial to mesenchymal transition during pancreatic cancer progression. *Carcinogenesis* 2008;**29**(2):252–62. doi:10.1093/carcin/bgm249.

[88] Turley RS, Finger EC, Hempel N, How T, Fields TA, Blobe GC. The type III transforming growth factor-β receptor as a novel tumor suppressor gene in prostate cancer. *Cancer Res* 2007;**67**(3):1090–8. doi:10.1158/0008-5472.CAN-06-3117.

[89] Hempel N, How T, Dong M, Murphy SK, Fields TA, Blobe GC. Loss of betaglycan expression in ovarian cancer: role in motility and invasion. *Cancer Res* 2007;**67**(11):5231–8. doi:10.1158/0008-5472.CAN-07-0035.

[90] Lee JD, Hempel N, Lee NY, Blobe GC. The type III TGF-β receptor suppresses breast cancer progression through GIPC-mediated inhibition of TGF-β signaling. *Carcinogenesis* 2010;**31**(2):175–83. doi:10.1093/carcin/bgp271.

[91] Wang L, Lau JS, Patra CR, Cao Y, Bhattacharya S, Dutta S, Nandy D, Wang E, Rupasinghe CN, Vohra P, et al. RGS-GAIP-interacting protein controls breast cancer progression. *Mol Cancer Res* 2010;**8**(12):1591–600. doi:10.1158/1541-7786.MCR-10-0209.

[92] Bhattacharya S, Pal K, Sharma AK, Dutta SK, Lau JS, Yan IK, Wang E, Elkhanany A, Alkharfy KM, Sanyal A, et al. GAIP interacting protein C-terminus regulates autophagy and exosome biogenesis of pancreatic cancer through metabolic pathways. *PLoS One* 2014;**9**(12):1–20. doi:10.1371/journal.pone.0114409.

[93] Wu D, Haruta A, Wei Q. GIPC1 interacts with MyoGEF and promotes MDA-MB-231 breast cancer cell invasion. *J Biol Chem* 2010;**285**(37):28643–50. doi:10.1074/jbc.M110.107649.

[94] Aigner A. Nonviral *in vivo* delivery of therapeutic small interfering RNAs. *Curr Opin Mol Ther* 2007:345–52 August 1,.

[95] Höbel S, Aigner A. Polyethylenimines for SiRNA and MiRNA delivery *in vivo*. *Wiley Interdiscip Rev Nanomed Nanobiotechnol* 2013:484–501 John Wiley & Sons, Ltd September 1. doi:10.1002/wnan.1228.

[96] Höbel S, Koburger I, John M, Czubayko F, Hadwiger P, Vornlocher HP, Aigner A. Polyethylenimine/small interfering RNA-mediated knockdown of vascular endothelial growth factor *in vivo* exerts anti-tumor effects synergistically with Bevacizumab. *J Gene Med* 2010;**12**(3):287–300. doi:10.1002/jgm.1431.

[97] Ibrahim AF, Weirauch U, Thomas M, Grunweller A, Hartmann RK, Aigner A. MicroRNA replacement therapy for MiR-145 and MiR-33a is efficacious in a model of colon carcinoma. *Cancer Res* 2011;**71**(15):5214–24. doi:10.1158/0008-5472.CAN-10-4645.