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VIP/PACAP, and their receptors and cancer

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Abstract

Purpose of review—To summarize the roles of VIP/PACAP and their receptors(VPAC1, VPAC2/PAC1) in human tumors as well as their role in potential novel treatments.

Recent findings—Considerable progress has been made in understanding of the effects of VIP/PACAP on growth of various tumors as well as in the signaling cascades involved, especially of the role of transactivation of the Epidermal growth factor(EGF) family. The overexpression of VPAC1/2, PAC1 on a number of common neoplasms (breast, lung, prostate, CNS, neuroblastoma) is receiving increased attention both as a means of tumor imaging the location and extent of these tumors, as well as for targeted directed treatment, by coupling cytotoxic agents to VIP/PACAP analogues.

Summary—VIP/PACAP has prominent growth effects on a number of common neoplasms, which frequently overexpressed the three subtypes of their receptors. The increased understanding of their signaling cascades, effect on tumor growth/differentiation, and the use of the overexpression of these receptors for localization/targeted cytotoxic delivery, are all suggesting possible novel tumor treatments.

Keywords

VIP; PACAP; cancer; lung cancer; neuroblastoma; glial tumors

Introduction

Vasoactive-intestinal-peptide(VIP), a 28-amino acid-peptide, [1] has sequence homology(67%) with pituitary-adenylate-cyclase-activating-polypeptide(PACAP)-27[2, 3••]. Their biologic actions are mediated by three classes of G-protein-coupled receptors(GPCR) [VPAC1, VPAC2, PAC1) which are members of the class II/class-B secretin-like receptors[3-5]. Other family members include glucose-dependent-insulinotrophic-peptide(GIP), glucagon, glucagon-like peptide-1, glucagon-like peptide-2, peptide-histidine-

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Conflicts of Interest

None

methionine/valine/isoleucinamide(PHM/PHV/PHI), growth-hormone-releasing factor(GRF) and secretin[3••, 4-5]. VPAC1, VPAC2, PAC1 activation stimulates diverse signaling-cascades, which principally are mediated by adenylate-cyclase, stimulation of PKA/EPAC proteins, as well as by activation of phospholipase-C causing protein-kinase C stimulation and cellular-calcium changes(especially PAC1-activation) in many tissues[3••, 4-5]. Numerous PAC1-receptor splice-variants occur(both NH2-terminal and intracellular-loop-3 variants) and these can differ in their signaling-cascades [3••, 5-8]. At present the differential importance of the different PAC1-splice-variants in normal physiology/pathophysiological conditions remains unclear[6, 9].

Recently, a number of reviews have covered various aspects of their possible therapeutic use in diseases, as well as their roles in various physiological/ pathophysiological conditions. These include their roles in inflammatory disorders[10-14]; immune/autoimmune diseases[10, 11]; neurodevelopment/behavior[15-17]; neuroprotection/neurodegenerative disorders[18-23]; pulmonary diseases(asthma, etc.)[24]; sepsis[25]; learning/memory disorders[26]; CNS/neurological disorders[14, 21, 27-29]; diabetes[30, 31]; migraine[32, 33]; stress-responses/disorders[34, 35]; renal injuries[20] and general reviews of their signaling/ pharmacology as well their roles in physiology/pathophysiology[3••, 5, 6, 36, 37]. In this review the roles of VIP/PACAP in neoplastic processes, which has generally not been dealt with recently[38, 39], will be concentrated on. Particular attention will be paid to VIP, which has received the most attention, both in its effects and signaling in neoplastic growth, but also in its roles in tumor-imaging and possible therapeutic roles in neoplastic processes.

General: VIP-PACAP-structure, receptors, pharmacology

PACAP occurs in two biologically-active forms, PACAP-27 and a COOH terminally-extended-form, PACAP-38[3••, 6]. PACAP-27/38 are processed from a 176-amino-acid precursor(preproPACAP) with the PACAP-gene located on chromosome 18p11, consisting of 5-exons/4-introns[3••, 6]. VIP is 28-amino-peptide and is processed from a 170-amino acid precursor(preproVIP) which also yields peptide-histidine-methionine/valine, and is encoded by a gene located at chromosome 1p11, with 7-introns/6-exons[3••, 5].

VIP has high-affinity for VPAC1/VPAC2 receptors, whereas PACAP-27/38 are nonselective, having high-affinity for all three receptor-subtypes[3••, 4-5, 40•, 41]. The investigation of the role of these receptor-subtypes in physiological/pathophysiological conditions has been hampered due to difficulty in developing selective-ligands for each receptor-subtype. The are no potent non-peptide-antagonists for these receptors[3••, 4-5, 40•, 41]. In addition, whereas selective-peptide agonist/antagonists exist for VPAC-receptors, for the PAC1-receptor, no potent, selective antagonists exist[3••, 4-5, 40•, 41]. Furthermore, until recently the only selective, potent PAC1-agonist was maxadilan, a 61-amino acid-peptide isolated from sand-flies[42], which is difficult to synthesize and therefore, rarely used[3••, 40•]. Recently, a number of studies report PACAP-analogues with differing selectivity for the PAC1-receptor, as well as enhanced stability[40•, 43, 44]. A recent study[40•] reporting the greatest selectivity, involved synthesizing conformationally-restricted-PACAP-analogues. A number were identified[40•] which were biologically active, potent and selective for PAC1. The most selective-analogues were >75-fold PAC1-preferring over VPAC1 and >800 fold over

VPAC2[40•]. At present these new selective PAC1- agonists have not been investigated in physiological/pathophysiological conditions.

General: VIP. PACAP and receptors in tumors

VIP-immunoreactivity(VIP-IR) occurs in a number of tumors[45•] and VPAC1 is overexpressed, resulting in high densities, in numerous cancers including bladder, breast, colon, liver, lung, pancreatic, prostate, thyroid and uterus- cancer[39, 45•, 46, 47••, 48]. In contrast, VPAC2 has been less well-studied, but is present in gastric leiomyomas; thyroid, gastric/pancreatic adenocarcinomas; lung-tumors, various sarcomas and neuroendocrine-tumors[46, 48-50]. PACAP-IR occurs in colon, lung and prostate-cancers[45•, 51]. PAC1 is present in tumors of the brain, breast, colon, lung, neuroendocrine, pancreas, pituitary, prostate as well as neuroblastomas/pheochromocytomas[45•, 46]. These results suggest that VIP/PACAP may play important roles in the modulation of growth/differentiation of many human-tumors[45•, 52]. VIP stimulates growth of many tumors including breast, lung, pancreas, prostate, in addition to various CNS tumors(gliomas, astrocytomas, etc), [38, 45•, 53, 54•, 55-57], as well as having an inhibitory-effect on the growth of other tumors(retinoblastoma, renal cell[58, 59]. PAC1-activation also stimulates growth of a number of tumors including neuroendocrine, brain, breast, prostate, pancreatic, colon and lung[45•, 60•, 61-62]; differentiation of pheochromocytoma cells(PC-12)[63], and has a growth inhibitory effect on some neoplasmas(medulloblastoma, gliomas)[64, 65].

Imaging/targeted-delivery of neoplasmas using overexpression of VIP/VPAC-receptors

Overexpression of somatostatin-receptors(sst1-5) by neuroendocrine-tumors/other tumors(CNS, etc.) is currently widely used for their imaging and when combined with positron-emission-tomography(PET) and computed-tomography(CT), is the most sensitive localization methody[66]. Furthermore, when combined with sst-ligands conjugated to cytotoxic-agents such as ^{90}Yttrium or ^{177}Lutetium, VPAC/PAC1-overexpression can be used therapeutically in patients with advanced disease[66, 67••]. A similar approach is being investigated for number of tumors(breast, prostate, etc.) overexpressing other GPCR's(bombesin, chemokine, gastrin, etc)[68-71] including VPAC-overexpressing-tumors(especially breast, prostate) using various radiolabeled-VIP analogues[39, 47••, 72, 73]. Recent studies report successful tumor-localization using various radiolabeled-VIP-analogues for experimental studies in animals for cancers of colon [74-76], prostate[77] and breast [78]. Furthermore, studies in humans report localization using various radiolabeled-VIP-analogues for breast-cancer[78, 79], pancreatic-cancer[80, 81], intestinal adenocarcinomas[73], neuroendocrine-tumors[73, 82] and colorectal-cancer[83]. In one study[80] no imaging was seen with a biological, active, radiolabeled-VIP-analogues in patients with pancreatic, colorectal-adenocarcinomas or neuroendocrine-tumors, and in vitro autoradiography demonstrated these tumors did not overexpress VPAC1[80].

Furthermore, a number of studies have used the overexpression of VPAC/PAC1-receptors on tumors to deliver-cytotoxic-agents[84-86, 86-89]. One novel approach coupled nanoparticles to VIP to target the VPAC-overexpression on various cancer-cells[84-85•, 86-88]. The nanoparticles are with various cytotoxic-agents, which cause cell-death in different

cancers[85•, 86, 87, 88]. In the specific tumor sections below, results with a number of these studies will be discussed in more detail.

VIP/PACAP: Lung-Cancer

VIP-receptors occur in 60% of lung-carcinoma-cells, with VIP-IR, pro-VIP-forms and COOH-terminal-extended forms frequently found[39, 45•, 46]. VPAC1/VPAC2 mRNA is found 51% and 48% of lung-cancer surgical specimens[48]. A number of recent basic-science and animal-studies provide insights into the signaling-cascades and effects of VIP on lung-cancer-cells, that may yield therapeutic approaches.

SCLC is a lung-cancer with endocrine-features, which kills approximately 25,000 US citizens annually. SCLC is treated with chemotherapy/radiation therapy[90]. This therapy is initially effective, but relapses occur and the median survival-time is <1 year. Addition of VIP to SCLC-cells stimulates secretion of bombesin(BB)-like peptides in a cAMP-dependent-manner[91]. Both VIP- and PACAP-receptor antagonists inhibit the growth of SCLC-cells[45•, 92-94]. A lipophilic, VIP-analog which functions as an antagonist(Stearyl-Nle(17)-neurotensin(6-11)VIP(7-28)[N-stearyl, Nle17)VIP hybrid][93], inhibited lung-cancer growth and had synergistic inhibitory-effects with chemotherapeutic agents. These results raise the possibility this approach could be of clinical value[94].

Recent studies demonstrate many G protein-coupled-receptors stimulate tumor growth by transactivating the epidermal-growth-factor-receptor(EGFR)[95••]. Recent studies on NSCLC-cells demonstrate PACAP-stimulates their growth by transactivating EGFR[55]. Transactivation requires phospholipase-C, not adenylate-cyclase, but stimulation of matrix-melloproteinases, Src-kinases, TGF-alpha release and generation of oxygen-free-radicals[55]. With other GPCR's(bombesin, neurotensin)[95••, 96••, 97] inducing growth in lung-cancer-cells, the simultaneous inhibition of the GPCR by an antagonist and a tyrosine-kinase-inhibitor(gefitinib, etc) leads to potentiated growth-inhibitory effects. These findings raise the possibility that a similar stragey could be consider with VIP/PACAP-receptors on these tumor-cells.

After binding of VIP to VPAC1, the VIP-VPAC1 complex is internalized to endosomes [3••, 4-5]. This raises the possibility that the VIP-receptor can be used to target cytotoxic-agents to the tumor-cell overexpressing the receptor, in a similar matter to that used in radio-imaging studies. VIP-ellipticine is composed of VIP-coupled to ellipiticine(E), a topoisomerase-II inhibitor) and activates VPACs in NCI-H1299 nonsmall-cell-lung cancer-cells(NSCLC cells)[98] and an analogue, VIP-LALA-E, reduced cellular-viability[98]. It was proposed[98] that the VIP-LALA-E was metabolized in the lysosomes, releasing cytotoxic E into the nucleus of NSCLC cells, preventing unwinding of the DNA, impairing DNA-replication. This is another example of how the overexpression of VPAC1 by lung-cancer-cells could be used as a molecular-target for cytotoxic agents[45•].

VIP/PACAP: Breast-cancer

Breast-cancer-cells have VIP/PACAP receptors in up to 100% of cases[46] and have high densities of VPAC1 and its mRNA[39, 45•, 99]. VPAC1, VPC2, and PAC1 are reported in

breast-cancer tumors[39, 45•, 100]. A number of results support a prominent role for VPAC/PAC1 activation in breast-cancer growth. In various breast-cancer cell-lines, VIP and PACAP have been shown to activate adenylate cyclase, increase VEGF expression and secretion, stimulate growth and the effects on the various cells inhibited by either VPAC1 antagonists or PACAP(66-38), a low affinity, but specific PAC1 inhibitor[39, 45•, 52, 94, 101]. Furthermore, the addition of a VPAC inhibitor to various breast-cancer cell-lines, potentiated the ability of taxol to inhibit proliferation[94]. VIP stimulates transactivation of both EGFR and the related receptor, HER2/Neu in breast-cancer cell-lines[102, 103] and the addition of PAC1-siRNA inhibited the stimulatory effect of VIP on secretion of VEGF and the transactivation of EGFR and HER2/Neu.

The overexpression of VPAC-receptors on breast-cancer-cells has recently been used both to image breast-cancer-cells as well as to target therapeutic agents to them. In nude mice studies with breast-cancer xenografts(T47D human breast-cancer-cells), ^{18}F-$^{15, 21}$Arg-VIP[104, 105] and ^{64}Cu-TP3982(VIP analog with agonist activity)[78] was reported to show enhanced localization to the cancer-cells. Subsequently, 4 synthetic analogues of VIP and PACAP [TP3939, TP3982, TP4200, TP3805], which had high affinities for VPACs and PAC1-receptors(Kd-0.72-3.3 nM), were labeled with ^{64}Cu and which showed enhanced binding to human breast-cancer tumor cells[106]. Current imaging modalities miss up to 30% of breast-cancer and do not distinguish benign from malignant tumors[107], therefore more sensitive imaging methods are needed. In a study in MMTVneu mice, which spontaneous develop breast-cancers, which resemble human breast-cancers in overexpressing VPAC-receptors, the efficacy of ^{64}Cu-VIP, was compared to CT scanning and ^{18}F-PET scanning[107]. ^{64}Cu-TP3805 identified all malignant tumors that overexpressed VPAC, was more sensitive than the other imaging modalities and did not identify the benign tumors that did not overexpress VPA receptors[107]. This result lead the authors to propose that PET scanning with this radiolabeled VIP analogue has potential for use in patients with early or metastatic breast-cancer. studies in humans, ^{99M}Tc-labeled VIP[79] identified tumors in 5 patients with breast-cancer and their was good concordance with results of CT and MRI localization.

To target breast-cancer-cells camptothecin(CPT, a topoisomerase I inhibitor) was coupled to a VIP analog(Ala$^{2, 8, 9, 19, 24, 25, 27}$, Nle^{17}, Lys^{28})VIP, [(A-NL-K)VIP] and the resulting(A-NL-K)VIP-L2-CPT was cytotoxic for MCF-7 breast-cancer-cells[108]. In this study[108] it was proposed that the(A-NL-K)VIP-L2-CPT was metabolized in the lysosome and the CPT was released which inhibits the unwinding of DNA and its subsequent replication. Many of the drugs used to treat breast-cancer have limited solubility, and to overcome this, long-circulating, sterically stabilized phospholipid micelles(SSMs) have been developed which function as Nano-size drug carriers[85•]. SSMs containing paclitaxel or 17-allylamino-17-demethyoxy-geldanamycin(an inhibitor of heat shock protein 90) have been targeted to breast-cancer-cells by coupling to VIP and shown to be cytotoxic[86-88, 109]. Breast-cancer stem cells(CSCs), which are important in tumor initiation, propagation, regeneration and resistance to conventional therapies[85•], overexpress VPAC-receptors[85•] and this has been used to target cytotoxic agents to them. In this study[85•] a novel approach was used with SSMs containing curcumin(a natural polyphenol with anti-proliferative, cytotoxic and

anti-metastatic effects in breast-cancer) which were conjugated to VIP, and were demonstrated to have enhanced cytotoxicity in breast-cancer-cells[85•].

VIP may be a promoter of breast carcinogenesis. Rat breast-cancer tissue in N-methyl nitrosourea treated rats had a higher density of VPAC1 than did normal breast tissue[110]. In C3(1)SV40T antigen mice, which spontaneously develop mammary cancer, VPAC1 immunoreactivity was detected[98]. In receptor binding studies specific ^{125}I-VIP binding was inhibited by(Lys^{15}, Arg^{16}, Leu^{27})VIP$^{1-7}$GRF$^{8-27}$ but not RO25-1553 suggesting that VPAC1 binding sites were abundant. Administration of a VPAC antagonist to the mice reduced the mammary tumor burden and altered the proteomic profile in the mammary tumors[98]. Retinoic acid, a chemo-preventive agent, down regulates VPAC1 expression in breast and lung cancer-cells[111, 112].

VIP/PACAP: Prostate-cancer

VIP/PACAP receptors are present in 88-100% of prostate cancers, each of the three subtypes are found in different studies, however the predominant subtype is VPAC1[46, 113]. In contrast to normal prostate tissue in which for the PAC1-receptor, the SV1/SV2 cassettes and the PAC1-null are the predominant isoforms present, in prostate-cancer the PAC1 null isoform predominates[114, 115].

Addition of VIP to prostrate cancer-cells stimulate cAMP generation; increases the transactivation of HER2/Neu, as well as the transactivation of the androgen receptor via a PKA-ERK mechanism and increases the expression of c-Fos, VEGF and COX-2, the latter dependent on activation of EPAC, NF-kB, PI3K and ERK[116•, 117-121]. VIP has a carcinogenic potential as it induces the malignant transformation of the human prostate non-tumor epithelial cell-RWPE-1, with stimulation of MMP2, MMP9, cyclin D1; decreased E-cadherin-mediated-cell-cell adhesion, increased cell motility and cell proliferation[122]. Similarly, in prostate-cancer cell-lines[123, 124] VIP stimulates cell proliferation and invasiveness via a NF-kB mechanism; increases expression of cyclin D1, MMP-2 and MMP-9; increases expression of the angigenic factor VEGF and COX-2, decreases cell adhesion and E-cadherin; suggesting VIP may act as a cytokine in early metastatic stages of prostate cancer[123-125].

There is limited data only on the use of VPAC1 overexpression on prostate-cancer cell for tumor imaging. In one study[77] PET imaging for localization of prostate-cancer cell xenografts(PC3 cell) was reported. In this study[77] ^{64}Cu-TP3939(a VIP agonist analogue) was synthesized and the prostate-cancer xenografts demonstrated enhanced uptake in nude mice and the ratio of the uptake in tumors to normal tissue was 4-fold and 2.7-fold increased at 4 and 24-hrs. Furthermore, in transgenic mice which spontaneously develop prostate cancer, ^{64}Cu-TP3939 imaged histological grade IV prostate intraepitheleal neoplasia, whereas 18F-FGD PET and CT scanning did not[77].

VIP/PACAP: Colon-cancer

VIP/PACAP-receptors are reported in 96% of colon adenocarcinomas[46] by autoradiography. In one study, 35% of well-differentiated colon-cancers had VPAC1,

whereas, 65% of moderately-differentiated tumors and 87% of poorly-differentiated tumors[126•]. Furthermore, in cancer-cells with highest overexpression of VPAC1, there was increased translocation of activated EGFR to the cell's cytoplasm[126•]. In addition, overexpression of VPAC1 in colon-cancer specimens is found in blood vessels surrounding the tumor and in associated macrophages[126•].

In a number of different colonic cancer cell-lines high affinity binding with VIP is seen[127, 128]. Activation of VPAC/PAC1-receptors on various colonic cancer-cells increases cyclic AMP, alters cellular calcium, activates chloride activated Ca2+ channels(HT29 cell)[129], stimulates ornithine decarboxylase activity[130] and stimulates growth[130, 131]. In other colonic cancer-cells VPAC-receptor activation inhibits invasiveness and growth[128, 132]. Inhibition of VPAC1-receptors inhibits colonic growth of some colonic cancer cell-lines[133].

Numerous studies demonstrate an important connection between inflammation and tumorigenesis, particularly in the case of colonic cancer[134]. The importance of VIP/PACAPs anti-inflammatory activities on the colonic tumorigenic process has been shown in recent studies[135, 136]. In PACAP-deficient mice(PACAP-KO mice)[135] treated with dextran sulfate sodium, an established model of colitis, the PACAP-KO mice developed more severe inflammation[135, 137], and 60% developed aggressive colorectal cancers, whereas none of the controls treated similarly developed colon cancers. APC mice, a model of spontaneous colon cancer, exhibit increased colonic inflammation and a defect in expression of both VIP and PACAP, which has led to the suggest this could be responsible for the increased proinflammatory enviroment in these animals and the increased initiation and progression of colonic cancers they develop[136].

The overexpression of VPAC-receptors by colon-cancer has been investigated both to image the tumors and for targeted therapy by coupling cytotoxic agents to VIP.

In one-study[83] colon-cancer patients, tumors were imaged using ^{123}I-VIP in 87% and 82% of patients with primary and relapsing cancers, respectively[83]. VIP scans were positive for 100% of the lymph node metastases and 89% of patients with liver metastases. However in another study, no imaging of colonic cancers could be seen, and this was attributed to the their low expression of VPAC-receptors[80]. ^{18}F-($R^{8, 15, 21}$, L^{17})VIP was used to localize C26 colon-cancer tumors in nude mice[74]. In nude mice bearing HT29 tumors, a 15-mer phosphorothioate anti-sense construct to the c-myc oncogene mRNA, which was conjugated to VIP-polylysine, strongly reduced colonic cancer xenograft proliferation[89].

VIP/PACAP: Central-nervous-system-tumors(CNS-tumors)

In various human gliomas(astrocytomas, epenydmomas, oligodendrogliomas), PAC1-receptors are present in 81-100%[138, 139] and in 20% of meningiomas[139]. PAC1 binding sites, but not VPAC sites, were found on glioma cell membranes[138]; PAC1-receptor mRNA was significantly overexpressed in glial tumors, especially in oligodendrogliomas[57] and PACAP-stimulated activation of adenylate in glial cell membranes[138, 139]. In various glioma cell-lines VPAC1 and VPAC2 receptors are also

found[140] with the VPAC1-receptor localized strongly to the nucleus, and there was a positive correlation between the degree of VPAC1 nuclear localization and glioma grade[140]. In two glioblastoma multiforme cell-lines[MO59K, MO59J][141] activation of VPAC and PAC1-receptors inhibited cell migration, but had no effect on cell proliferation. Furthermore, increasing expression of VPAC-receptors resulted in decreasing levels of cell invasion in these glioblastoma cell-lines and this inhibitory action was found to be mediated by inhibition of AKT signaling[142•]. Furthermore, in three other glioblastoma multiforme cell-lines(U87, U118, U373), PAC1, but not VPAC-receptors were found[143], and PACAP activated adenylate cyclase in these cells, as well as increased cytosolic Ca^{2+}, and a PAC1 antagonist inhibited proliferation of each of the three glioblastoma multiforme cell-lines[143].

In childhood, primitive neuroectodermal tumors(medulloblastomas, etc.) are one of the most frequent groups of CNS malignant tumors[144]. In one study[144], 86% of the central primitive neuroectodermal tumors had VPAC1 mRNA and 75% VPAC2 mRNA. Furthermore, the addition of VIP to 10 primitive neuroectodermal tumor cell-lines inhibited growth in 70%[144]. These tumors also possess PAC1-receptors[64, 145]. In studies of mutant mice exhibiting an enhanced development of medulloblastomas, generally hedgehog signaling and PKA play opposing roles[145, 146•]. Deletion of single copy of the PACAP gene in these mutant mice((ptc1 mice) results in 2.5 fold increase in the development of medulloblastomas, demonstrating that PACAP exerts a powerful inhibitory effect, which is PKA-dependent, on the induction, growth or survival of this tumors[145]. Primary tumor-spheres made from these medulloblastomas, exhibited constitutive hedgehog signaling[64, 145]. The addition of PACAP to these medulloblastoma primary tumor-spheres antagonized the hedgehog signal, which was blocked by a PKA inhibitor, and PACAP- inhibited proliferation, suggesting that the regulation of hedgehog signaling in these tumor by PACAP administration might proved a novel therapy[64].

Whereas a number of other studies have also reported activation of PAC1 or VPAC1-receptors on various glial cell-lines inhibits their proliferation, other studies report activation of these receptors on these tumor cells increase growth[54•, 65, 147, 148].

VIP/PACAP: Neuroblastomas

Neuroblastoma(NB), an embryonal-tumor arising from the sympathetic- nervous-system, is the most frequent neonatal malignancy and is also common in early childhood. Almost all NB-tumor-cells possess at least one of the VPAC/PAC-receptors, with VPAC1-receptors reported in 60-100%, VPAC2 in 31% and 9AC1 in 60-100% [149-151]. In addition, PACAP-mRNA is frequently detected in NB-cells(60-100%)[149, 151], whereas VIP-mRNA is less frequetly detected(12-75%)[149, 151]. In a study of six NB-tumor-cell-lines, PAC1-receptors were detected in the highest amounts and was present in 100% of the cell-lines, whereas VPAC2/VPAC1 were present in low amounts in 4/6(66%) and 2/6(33%) of the NB-cell-lines[152]. Detailed studies of one NB-cell-line(SH-SY5Y- cells) demonstrated that multiple different PAC1-receptor splice-variants are present involving the 3rd intracellular-receptor domain(ic3) as well as the amino-termial-receptor domains(N-terminal)[152]. The null-ic3 splice-variant was the predominant form found[152]. Different PAC1-splice-variants

can respond with different potencies, affinities, efficacies to VIP/PACAP[7], and this was seen with the different different SH-SY5Y- splice-variants[152]. This variation in the expression of different PAC1-splice variants is reported to be a mechanism of fine tuning of brain activity[153•], however, what role it plays in different NB-tumor-cells is unclear.

In NB-cells as well as NB-cell-lines, PACAP activate phospholipase-C[152], and stimulates VIP-gene-transcription[154, 155] and c-Fos expression[155]. PAC1 stimulation of c-Fos/VIP-expression is dependent on PAC1-activation of PKA, PKC/ERK1/2[155]. Furthermore, PAC1 stimulation of c-Fos requires calmodulin[156]. Both PACAP/VIP can activate adenylate-cyclase in various NB-tumor-cells[150, 152, 154].

VIP/PACAP can have a marked effect on the growth/differentiation of various NB-tumor-cells. VIP/PACAP stimulate growth/proliferation of NB-tumor-cells[157, 158] and induce differentiation and neuritogenesis[158, 159]. The neurotrophic-effects of PACAP on the NB-tumor-cell, SY5Y are mediaited by cAMP dependent processes involving Epac-dependent activation of ERK and activation of the p38 kinase[50]. Furthermore, 22% of NB-tumors overexpress MYCN and this is associated with a poor-prognosis[160]. VIP-treatment of NB-tumor-cells in vitro[160] decreases by 25% MYCN-expression and it has synergistic effects with retinoic-acid, which is used in the theapy of NBs[160]. This result suggests that VIP in combination with retinoic-acid may have potenital benefit in patients with NBs overexpressing-MYCN[160].

VIP/PACAP: Pancreatic-cancer

Pancreatic-ductal-cancer frequently express VIP/PACAP-receptors(65%)[46].

VPAC1mRNA is present in human pancreatic-cancer cell-lines such as CAPAN-2[161]. VIP increases cAMP/c-fos mRNA in CAPAN-2 cells which is antagonized by VPAC1-inhibitors[161]. Furthermore, proliferation of CAPAN-2-cells and tumors in nude mice is inhibited by VPAC1-inhibitors[161].

VIP-hypersecretion by neural-tumors such as ganglioblastomas/neuroblastomas in children or non-β cell pancreatic-tumors(VIPomas), causes a characteristic clinical syndrome(Verner-Morrison-syndrome)[162]. VIPomas cause large-volume, watery-diarrhea, hypokalemic and achlorhydric(WDHA)[162]. A recent study demonstrates VIPoma-cells express both VPAC1 and VIP-IR, raising the possibility the hypersecreted VIP could be having an auto-regulator effect on the tumor-cells[163].

NB-tumors occasionally hypersecrete VIP causing watery diarrhea and the VIPoma syndrome[164]. These occur in children, approximately 80-cases have been described, and it usually occurs in well-differentiated-NBs[164].

VIP/PACAP: Other tumors

A number of other tumors have also been described in which PACAP/VIP effect(stimulatory/inhibitory) their growth, but these are, in general, less well-studied than the tumors reviewed above and will not be covered in this review. These include pheochromocytomas[63, 165], gastric-cancer[166, 167•], cervical-cancer[168],

choriocarcinoma-cells[169], neuroendocrine-tumors[60•, 62, 170] and renal-cancer[59, 171•, 172].

Summary

A large number of different neoplasms possess and overexpress receptors for VIP/PACAP. In some tumors activation of these receptors have growth stimulatory effects and in others they have growth inhibitory effects. Furthermore, in a number of different tumors VIP/PACAP have an autocrine function with the tumor both possessing VIP/PACAP receptors as well as secreting the active peptide.

Recent studies, reviewed here, are providing increased insights into the cellular signaling cascades mediating the growth effects of this family of receptors on both normal tissues and tumors. Particularly important in a number of cases is their ability to transactivate the EGF family of receptors.

The overexpression of VPAC/PAC receptors is receiving increased attention with many of the tumors, not only as a means to imaging their location and extent, but also as a method to target cytotoxic agents to these tumors This receptor family is receiving increased attention because of the success of using this approach with radiolabeled somatostatin analogue for a wide range of neuroendocrine tumors and because the VIP/PAC family of receptors are overexpressed on a number of common tumors that do not overexpress somatostatin receptors.

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Abbreviations

AP	activator protein
CT	computed tomographic scanning
EGFR	epidermal growth factor receptor
ERK	extracellular signal-regulated kinase
EC	extracellular
^{18}F-FGD	^{18}Fluoro-deoxyglucose uptake
GPCR	G-protein-coupled receptor
IC	intracellular
MMP	matrix metalloprotease
MEK	mitogen/extracellular signal-regulated kinase
NB tumor cell	neuroblastoma tumor cell

NSE	neuron specific enolase
PET scanning	positron emission tomographic scanning
PACAP	pituitary adenylate cyclase activating polypeptide
PHM	Peptide histidine methionine
PL	phospholipase
PK	protein kinase
SCLC	small cell lung cancer
SV	splice variant
TGF	transforming growth factor
TKI	tyrosine kinase inhibitor
VEGF	vascular endothelial growth factor
VIP	vasoactive intestinal peptide

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KEY POINTS

- VIP and PACAP have prominent effects on growth (stimulatory or inhibitory) of a number of normal and neoplastic tissues and can function in an autocrine manner to affect growth
- Many common neoplasms overexpress receptors for one or more of the three receptors mediating the actions of VIP/PACAP (VPAC1, VPAC2, PAC1)
- The is increasing interest in using tumor overexpression of VIP/PACAP receptors for targeting tumors both for imaging and for receptor-mediated cytotoxicity (using nanoparticles, coupled to radiolabeled or cytotoxic compounds)
- This latter approach is receiving particular attention with cancers of the breast, lung, prostate, CNS, colon and with neuroblastomas.