

# Neurotransmitters and Chemokines Regulate Tumor Cell Migration: Potential for a New Pharmacological Approach to Inhibit Invasion and Metastasis Development

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**Abstract:** The migration of tumor cells is a prerequisite for tumor cell invasion and metastasis development, which accounts for over 90% of cancer mortality. Therefore a major focus of current tumor biological research is the study of those factors that regulate tumor cell migration. Those chemokines and neurotransmitters that bind to G-protein coupled receptors (also known as serpentine receptors) are the most prominent of these factors. Neurotransmitters have been identified that have not only a stimulatory (e.g. norepinephrine) effect, but an inhibitory effect (e.g. GABA) as well. This is an especially fortuitous development, because many known agonists and antagonists of neurotransmitter receptors are currently being successfully used in the treatment of other pathological conditions (e.g.  $\beta$ -blockers in the treatment of cardiovascular diseases). Likewise, chemokine receptor antagonists, which are under development for the treatment of HIV or rheumatoid arthritis, may be effective tools for the inhibition of chemokine-driven tumor cell migration as well. A further approach to inhibit tumor cell migration arises from the investigation of the relevant signal transduction pathways. The PKC alpha, for example, is a key enzyme in the regulation of tumor cell migration, but not of leukocyte migration. It thus offers a selective target opportunity for specific pharmacological agents to interfere with tumor cell migration. In this review we therefore summarize the current findings on those serpentine receptors involved in the neurotransmitter- and chemokine-regulated tumor cell migration, on the underlying signal transduction pathways, and on the opportunities to inhibit tumor cell migration and ultimately metastasis development with pharmaceutical agents.

**Key Words:** Tumor cell, migration, metastasis, chemokines, neurotransmitters, serpentine receptors, signal transduction.

## INTRODUCTION

Cancer is the second leading cause of death in industrial countries. Although oncology has made significant progress in the prevention and treatment of cancer, a third of the population of the industrial countries will develop this disease at some point in their lives, and a quarter will eventually die from it. Surgery and chemotherapy are the most effective tools in the treatment of cancer, often leading to a successful treatment for incipient tumors. Cancer begins as a localized disease, and is characterized by at least six acquired capabilities [1]. Five of them contribute to the growth of a tumor: these are the self-sufficiency in growth signals, the evasion from apoptosis, a sustained angiogenesis, a limitless replicative potential, and an insensitivity to antigrowth signals. Solely the sixth capability, tissue invasion and metastasis, leads to the dissemination of the tumor cells within the body. This is, however exactly the key event, which makes cancer the fatal disease that it is. Consequently over ninety percent of the cancer patients do not die from the primary tumor, but from the development of metastases.

## PROMIGRATORY EFFECTS OF CHEMOKINES AND NEUROTRANSMITTERS

Active migration is an inherent and essential ability of tumor cells to invade surrounding tissues and to disseminate

to distant organs. Tumor cells share this ability with a few specialized cells in the adult organism, most prominently leukocytes, fibroblasts, and stem cells. The migratory activity of all these cells is regulated by ligands to a receptor class termed as serpentine receptors, G protein-coupled receptors or seven-helix receptors. Two major groups of ligands to these receptors are the chemokines and the neurotransmitters. Chemokines are predominantly known as regulators of leukocyte migration, integrally involved in the processes of inflammation, infection, and tissue injury [2]. Besides this, chemokines have been identified to influence a multitude of tumor cell functions including growth, angiogenesis, survival and migration [3], and functional similarities between cancer and inflammation have been discussed [4]. Chemokines do not only induce migration, but also deliver a localization signal for the development of metastases, as was shown by Muller and co-workers for the role of the stromal cell-derived factor (SDF)-1 in the development of breast cancer metastases in the mouse [5]. Furthermore, chemokines are produced and released by tumor cells and can act in an autocrine manner [6]. Balkwill and Mantovani deduced from such observations the hypothesis that tumors, whose cell functions (e.g. metastasis development) are driven by chemokines, might be appropriate targets for chemokine receptor antagonists [4]. These receptor antagonists are under development for other diseases such as the CXCR4 antagonist KRH-1636 in HIV [7], or CCR1 antagonists like CP-491, 715 in rheumatoid arthritis [8, 9] and in multiple sclerosis [10]. Although such strategies are very promising, the most critical pharmaceutically relevant difficulty in regard to the chemokine system

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is its redundancy. Much more ligands than receptors are known (about 50 ligands as compared to about 20 receptors [11]), and more than one ligand binds to a certain receptor (e.g. RANTES, Eotaxin, MCP-2/-3/-4 and other chemokines bind to CCR3). Therefore, treatment of a tumor with chemokine receptor antagonists might be a very effective tool in cancer treatment, but would have to be carefully monitored for side-effects on the immune system or other organ systems.

In the neurotransmitter system, the relationship between ligands and receptors is converse. One ligand binds to several receptor subtypes, which are differentially expressed in the body's tissues [12]. Similar to chemokines, neurotransmitters are regulators for cell migration in both leukocytes [13] and tumor cells [12]. Table (1) summarizes the effects of neurotransmitters on tumor cell migration and, where applicable or known, the receptor by which this effect is mediated. It is noteworthy that only few results are available on the expression of neurotransmitter receptors in tumor tissues. The following neurotransmitters have proven effects on tumor cell migration.

### Epinephrine and Norepinephrine

Catecholamines are the best understood neurotransmitters for their role in carcinogenesis and tumor progression. Catecholamines are released in a stress reaction and therefore are also known as stress hormones. Stress in turn is a major risk factor, not only for cardiovascular disease, but also for the development of cancer. Psychosocial factors like job-related stress have been implicated in tumor progression as early as 1926 [14]. Catecholamines are derivatives of the amino acid tyrosine; epinephrine and norepinephrine are the

prominent representatives in vertebrates. Presently 10 distinct adrenoreceptors have been characterized, subdivided into  $\alpha$  and  $\beta$  adrenoreceptor groups. Norepinephrine has been shown to strongly induce the migration of tumor cells [15, 16], and epinephrine is a modulator for the carcinogenesis in the lung of hamsters induced by the tobacco-specific nitrosamines [17]. These effects of norepinephrine and epinephrine in both carcinogenesis and migration can be inhibited by  $\alpha$ -adrenoceptor antagonists [16, 17]. This migratory effect is mediated via the  $\beta$ -adrenoceptor, suggesting the potential use of specific, non-heart-active  $\beta$ -adrenoceptor antagonists for the pharmacological prevention of metastasis development. Since norepinephrine is furthermore an inducer of directed, chemotactic migration of breast carcinoma cells [15], it is necessary to consider whether catecholamines are responsible not only for the initiation of tumor cell migration, but also for the directionality and thus for the localization of metastases. This hypothesis, drawn from experimental *in vitro* results, is supported by the clinical observation that certain tumors, such as the small cell lung carcinoma (SCLC), frequently develop metastases in the catecholamine-producing adrenal glands [18, 19], and in the brain [20, 21]. Furthermore, noradrenergic nerve fibers are found in the spleen, thymus, bone marrow, and lymph nodes [22], which may provide a chemoattractive source for tumor cells, as has been postulated previously by Brabletz and co-workers [23].

### Dopamine

Dopamine, a precursor in the synthesis of catecholamines, has also been characterized as a stress hormone. Dopamine is synthesized in the brain and other areas of the

**Table 1. Neurotransmitters with known Function on Tumor Cell Migration**

Neurotransmitter	Tumor cell type	Receptors engaged
<b>Stimulatory</b>		
Catecholamines	Breast, prostate and colon carcinoma	2-adrenoceptor [15, 16, 32]
Dopamine	Breast carcinoma	D2-R [32]
Histamine	Carcinoma and melanoma	H1R [34]
Substance P	Colon and breast carcinoma, SCLC	NK-1R [12, 15, 49]
Opioid peptides	Endorphin: SCLC [49] Enkephalin: breast carcinoma [15]	
Gastrointestinal neuropeptides	Bombesin/GRP: colon and prostate carcinoma [61, 62] CCK/Gastrin: pancreatic and colon cancer [65, 69] VIP: prostate cancer [61] (inh. for murine colon cancer [75]) Endothelin: Kaposi's sarcoma	ET(A/B)R [76]
Inflammatory neuropeptides	Bradykinin: prostate cancer CGRP: prostate cancer [61]	B1R [82]
<b>Inhibitory</b>		
GABA	Colon carcinoma	GABA <sub>B</sub> -R [85]
Anandamide	Colon carcinoma	CB1-R [89]

central and peripheral nerve systems [24], and has been implicated in the pathological conditions of schizophrenia and Parkinson's disease [25]. Moreover it elicits a promigratory effect in tumor cells, too [15]. There are five groups of known dopamine receptors, termed as D1- to D5-receptors [26]. In addition to nerve cells, the D2-receptor has been found on endothelial cells, lymphocytes, and tumor cells [27-31]. Metoclopramide is a selective inhibitor for the dopamine D2-receptor and is in clinical use for the treatment of nausea. We have observed that the D2-receptor is expressed in breast carcinoma cells, and that the promigratory effect of dopamine is inhibited by metoclopramide [32].

### Histamine

Histamine is a hormone released locally in the inflammatory response by mast cells, and is a neurotransmitter in the vertebrate and invertebrate brain. Four serpentine receptors are known for histamine, termed as H1R to H4R. H1R and H2R are mainly postsynaptically located, while the HR3 is exclusively presynaptically located [12]. HR4 is structurally and pharmacologically related to HR3, but primarily expressed in the bone marrow and on eosinophil granulocytes [33]. Histamine exerts chemoattractive function on human carcinoma and melanoma cells via the H1R [34], and increases the survival rate of patients with metastatic melanoma in combination with interleukin-2, as shown in clinical phase II and III trials [35, 36].

### Angiotensin

Angiotensin II is a potent vasoconstrictor and has an integral role in the aldosterone regulation. It is an octapeptide, which is activated by the enzymatic digestion of the decapeptide angiotensin I by the angiotensin-converting enzyme (ACE). Besides the above discussed  $\beta$ -blockers, inhibitors of the ACE represent a second group of pharmacological substances in use for the regulation of blood pressure in hypertension and congestive heart failure. Captopril, an ACE inhibitor, is in clinical use for the aforementioned indications, and has also been shown to be a potent inhibitor of neovascularization [37]. Furthermore, direct blockade of the angiotensin II type 1 receptor (AT1), one of the two known angiotensin II receptors, inhibited tumor angiogenesis and led to a reduced growth of melanoma cells engrafted in mice [38]. Accordingly, Lever and co-workers found in a retrospective cohort study based on 5207 patients that the long-term use of ACE inhibitors may protect against cancer [39]. With regard to cell migration, angiotensin II is an inducer for the migration of monocytes [40] and is chemotactic for T lymphocytes [41]. However, to our knowledge, the role of angiotensin II in the regulation of tumor cell migration has not been under investigation so far.

### Substance P

Substance P is a peptide of the neurokinin family, localized in the central and peripheral nerve systems [42]. Substance P plays a role in the regulation of affective behaviour, in stress reactions, and in anxiety and depression [42, 43]. Pharmacological inhibition of the neurokinin-1 receptor, the preferential receptor for substance P, is an

effective tool for the treatment of depressive disorders [44]. Furthermore, the lung is richly supplied with nerves that secrete substance P [45], and this neurotransmitter might contribute to the pathogenesis of asthma, because of its inflammatory effects on the airways [46]. Substance P also influences the migration of neutrophil granulocytes across endothelial and subendothelial barriers towards inflammatory sites of the lung, thereby regulating their interstitial accumulation and traffic to the alveolar space [47, 48]. Substance P induces the migration of colon [12] and breast [15] carcinoma cells and is a chemoattractant for SCLC [49]. In breast carcinoma cells, the promigratory effect is mediated via the neurokinin (NK)-1 receptor, as we have shown by the use of the specific receptor blocker L-733, 060 [32].

### Opioid Peptides

Proopiomelanocortin (POMC) is the neuropeptide precursor for the opioid peptides  $\delta$ -endorphin, enkephalins and dynorphins. The active neuropeptides are generated from the 91 amino acid molecule POMC by post-translational cleavage, and their function ranges from influences on affective behaviour to analgesia. The POMC gene is not only expressed by nerve cells, but by leukocytes as well [50]. Thus, the immune system plays a major role in the control of pain [51, 52]: immune cells release the opioid peptides in inflamed tissue, which act on opioid receptors of peripheral sensory nerve endings [53], and in contrast, proinflammatory cytokines such as interleukin-1 and -6 are critical mediators of exaggerated pain, which is mediated by the substance P, glutamate, or fractalkine [54, 55]. With regard to cell migration, met-enkephalin induces migration in breast carcinoma cells [15], and  $\delta$ -endorphin is a chemoattractant for SCLC [49]. Met-enkephalin binds to the  $\delta$ -opioid receptor ( $\delta$ -OR or DOR), and with ten-fold lower affinity to the  $\mu$ -opioid receptor ( $\mu$ -OR or MOR), while  $\delta$ -endorphin is equiactive on both these receptors. Both ligands, met-enkephalin and  $\delta$ -endorphin, have low affinity for the  $\kappa$ -opioid receptor [56]. Which of these opioid receptors is responsible for the observed promigratory effects is not yet clear.

### Gastrointestinal Neuropeptides

Four neuropeptides with gastrointestinal function have so far been identified to have a regulatory function in tumor cell migration. The gastrin-releasing peptide (GRP) is the mammalian homologue of a peptide isolated from the frog *Bombina bombina*; hence this substance is called bombesin. Bombesin/GRP exert direct effects on tumor cell migration, and mediate the release of gastrin and cholecystokinin (CCK) from gastrointestinal endocrine cells of the stomach and upper small bowel, respectively. Gastrin and CCK have effects on tumor cell migration, too. Receptors for bombesin/GRP are expressed in tumors from various tissues including the colon [57], prostate [58], breast [59] and lung [60]. In colon and prostate carcinoma, bombesin has been shown to increase the invasive potential *in vitro* [61, 62]. Gastrin and CCK bind to two related receptors, which are differentially expressed in the gastrointestinal tract. The CCK-A receptor (CCK-AR) is found in pancreatic acinar cells, while CCK-BR is predominantly expressed in the stomach [63]. The CCK-AR has a much higher affinity for gastrin than the CCK-BR [64]. CCK-BR is expressed in

adenocarcinomas of all areas of the gastrointestinal tract [65-67]; in contrast, CCK-AR is rarely expressed in gastroenteropancreatic tumors [68]. CCK regulates the invasiveness of human pancreatic cancer cell lines [69], and glycine-extended gastrin promotes the invasiveness of human colon cancer cells [65]. Besides these two important gastrointestinal neuropeptides, two further neurotransmitters will be discussed here, which have a known effect on tumor cell migration. The first is the vasoactive intestinal polypeptide (VIP). It is a large neuropeptide 28 amino acids in length, and is expressed in gastrointestinal organs, in the lung and the prostate [70]. Receptors for VIP are expressed in breast cancer [71] and tumors of other tissue origin [72]. Reports on the role of VIP in the regulation of leukocyte and tumor cell migration are ambiguous. VIP inhibits T lymphocyte migration [73], but stimulates the migration of eosinophil granulocytes [74]. Furthermore, migration of the prostate cancer cells was increased by VIP [61], but the migration of murine colon carcinoma cells was inhibited, as was reported earlier by the same group [75]. The last gastrointestinal neuropeptide to be discussed is endothelin. Endothelin regulates gastrointestinal function, but is also known to play a role in the regulation of arterial and pulmonary blood pressure and in cardiovascular diseases. There are four closely related forms of endothelin (ET) known. ET-1 is overexpressed in Kaposi's sarcoma lesions and promotes invasiveness and tumor growth via both known ET receptors, ETA-R and ETB-R, in an autocrine way [76]. Furthermore, colon cancer cells express higher levels of endothelin receptors than normal colon epithelium [77], and plasma ET-1 levels are increased in patients with colorectal cancer [78], suggesting a similar autocrine function in this type of cancer, too.

### Inflammatory Neuropeptides

Bradykinin and the calcitonin gene-related peptide (CGRP) are not structurally related to each other, but both play a role in inflammatory processes, and are therefore called inflammatory neuropeptides [79, 80]. Bradykinin exerts its proinflammatory effect via the activation of the constitutively expressed B2 receptor, while the B1 receptor is activated by a biological active metabolite of bradykinin. The B1 receptor is underexpressed in normal tissues and upregulated during inflammatory responses [81]. In human benign and malignant prostate specimens B2R is ubiquitously expressed, but the B1R is detected only in prostatic intraepithelial neoplasia and malignant lesions, and not in benign prostate tissues [82]. Specific stimulation of endogenous B1R promotes growth, migration, and invasion of PC-3 prostate cancer cells [82]. Few results are available for CGRP, but the data on its function in cell migration show a function similar to bradykinin: CGRP is a chemotactic mediator for T lymphocytes [80] and has a promigratory effect on neutrophil granulocytes [83], and on the prostate cancer cell line PC-3 [61].

### INHIBITORY NEUROTRANSMITTERS

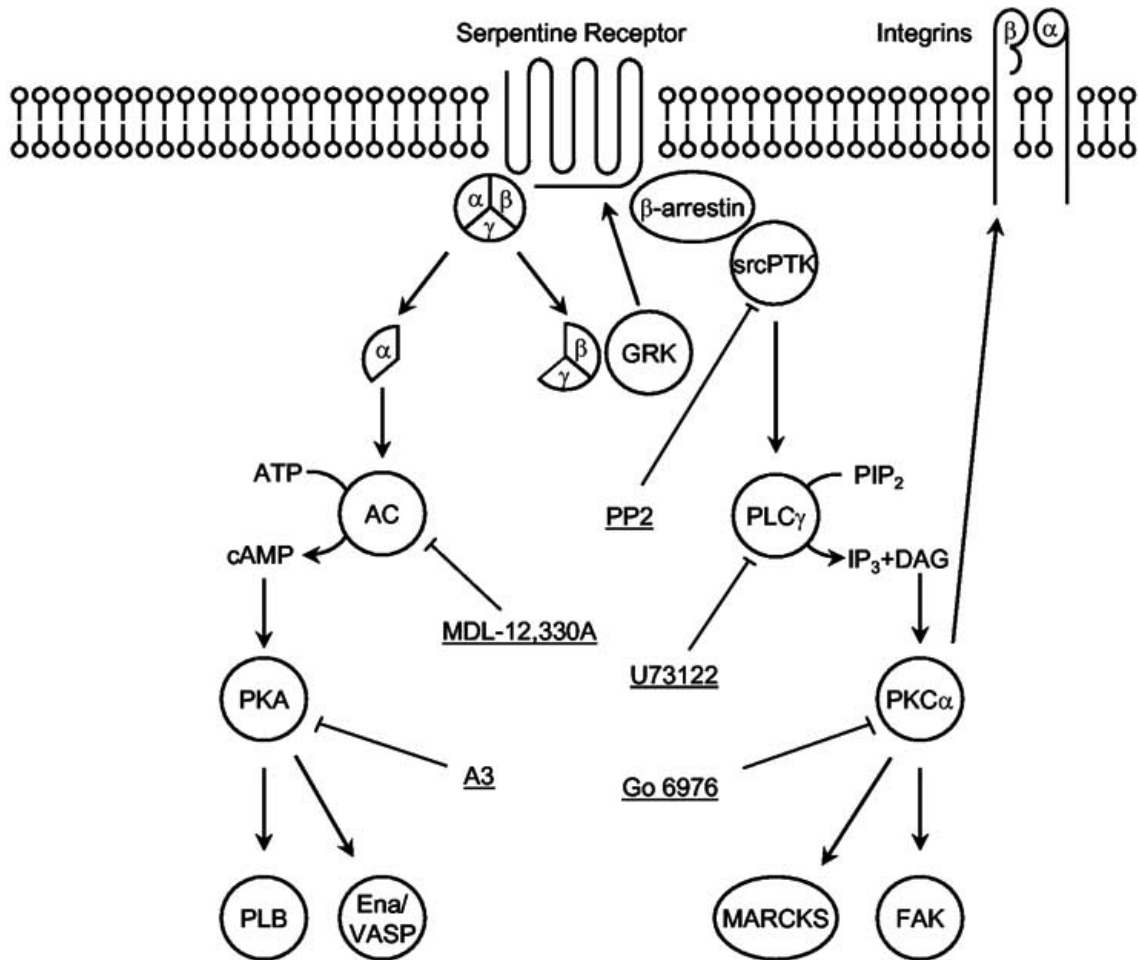
Besides the above discussed promigratory effects of chemokines and neurotransmitters on the migration of tumor cells, there is increasing evidence that both groups of these

serpentine receptor ligands also have members that exert inhibitory function. In neutrophil granulocytes we have shown that interleukin-8 can function as an inhibitor for the migration induced by bacterial peptides [84]. The unique feature of this effect is the change of migratory characteristics: while the bacterial peptide leads to a recruitment of cells for migration, interleukin-8 does not deliver an ultimate stop-signal, but reduces the migratory activity of each single cell by an increase of the length of pauses between phases of migratory activity [84].

Gamma-aminobutyric acid (GABA) inhibits the norepinephrine-induced migration via the GABA<sub>B</sub>-receptor in tumor cells [85]. Baclofen is a GABA<sub>B</sub>-receptor agonist, which is in use for the treatment of epilepsy. This agonist not only inhibits the norepinephrine-induced migration of colon carcinoma cells [85], but has also been shown to reduce gastric and colon carcinogenesis when systemically administered in rats [86, 87]. Thus, GABA agonists might be useful pharmacological agents in the treatment of cancer, as has been discussed by Ortega [88]. A similar inhibitory effect on the norepinephrine-induced colon carcinoma migration was recently observed for anandamide, the endogenous ligand for cannabinoid receptors (CB-R) [89]. Here, the most interesting point is that tumor cell migration is inhibited via the CB-1 receptor, while the chemokine-induced migration of T lymphocytes is inhibited via the CB-2 receptor, and the migration of neutrophil granulocytes is not affected at all. This would allow a specific inhibition of tumor cell migration using selective CB-1 receptor agonists, whereas the function of the immune system would not be affected.

### THE SIGNAL TRANSDUCTION OF SERPENTINE RECEPTORS

On the intracellular side, serpentine receptors are coupled to heterotrimeric G proteins [90]. With regard to tumor cell migration, there are two important signaling pathways, which are activated by these G proteins, as is illustrated in Fig. (1). Upon activation, the G protein disengages into a GTP-bound subunit and a subunit, each of these parts is signaling in an independent route [90]. The adenylyl cyclase is a key target molecule of the subunit. Depending on the type of receptor, stimulatory (G<sub>s</sub>) proteins or inhibitory (G<sub>i</sub>) proteins are activated, which increase or inhibit, respectively, the enzymatic activity of the adenylyl cyclase and thereby regulate the production of cyclic adenosine-monophosphate (cAMP) [90]. For example, the norepinephrine-binding -adrenoceptors are coupled to G<sub>s</sub> proteins and have a promigratory effect, while the GABA<sub>B</sub>-receptor and the cannabinoid receptors are coupled to G<sub>i</sub> proteins and their ligands, GABA and anandamide, respectively, have an inhibitory effect on migration [85, 89]. For angiotensin II, two receptors are known, which are both coupled to G<sub>i</sub> proteins [91], and angiotensin II is known to inhibit the adenylyl cyclase [92]. From these results it is reasonable to assume that angiotensin II has an inhibitory effect on angiotensin II-receptor positive tumor cells. The protein kinase A (PKA) is activated by cAMP and has a plethora of downstream targets involved in the regulation of migration. For example, the PKA is involved in the regulation of the cytosolic calcium concentration via phospholamban (PLB)



**Fig. (1).** Schematic overview of signal transduction pathways involved in the regulation of tumor cell migration, and examples for a selective experimental inhibition (underlined).

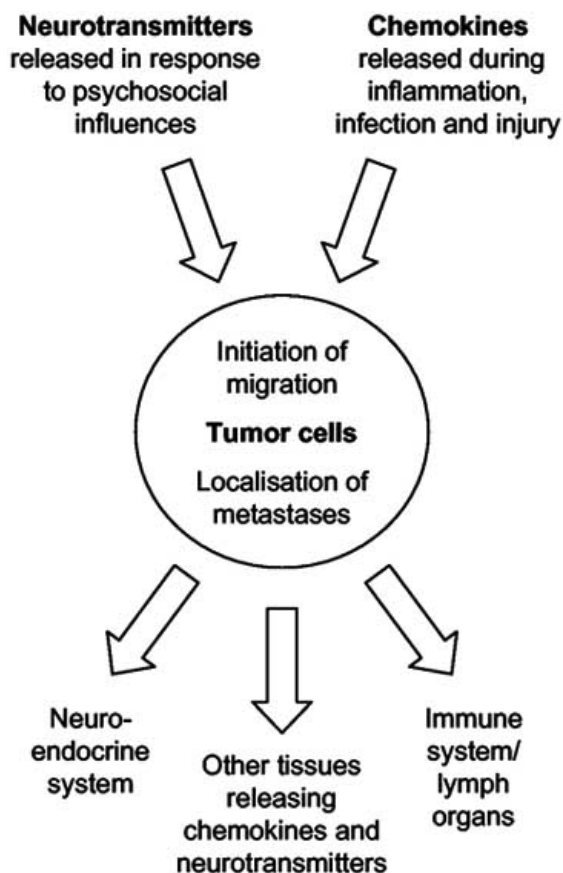
and the sarcoplasmic/endoplasmic calcium ATPase (SERCA) [85], and in the regulation of the actin assembly/disassembly via action on Ena/VASP proteins [93].

The second pathway, which is activated by the  $\beta\gamma$  subunit of the G proteins, is the same in either case, regardless whether the receptors are coupled to  $G_s$  or  $G_i$  proteins; Fig (1). This  $\beta\gamma$  part of the G proteins activates G protein-coupled receptor kinases (GRK) [94], which engage src-protein tyrosine kinases (srcPTKs) via  $\beta$ -arrestin [95]. A downstream target molecule of these tyrosine kinases is the phospholipase C (PLC). The PLC catalyzes the breakdown of phosphatidyl-inositolbisphosphate to the metabolites diacylglycerol, an activator for the protein kinase C (PKC), and inositol-1, 4, 5-phosphate, a second messenger which opens intracellular calcium channels [16]. The PKC is a central regulatory molecule in the migration of tumor cells; the migratory activity of a tumor cell line depends on its level of expression and inhibition of this enzyme completely abolishes migratory activity [96]. Most importantly, the direct link of the PKC to  $\beta_1$  integrins is crucial for a chemotactic migration of tumor cells [97]. Furthermore, the PKC phosphorylates the focal adhesion kinase (FAK) [98], and actin-regulating proteins such as the myristoylated alanine-rich C kinase substrate (MARCKS) [99].

Various selective inhibitors for each component of these signal transduction pathways are available and widely in experimental use. Examples for experimental inhibitors are shown in Fig. (1). However, none of these pharmacological inhibitors has yet been clinically tested for the inhibition of metastasis development, but promising results have been obtained with selective enzyme inhibitors, e.g. with regard to the treatment of chronic myeloid leukemia. In over 90% of patients with this disease, a reciprocal chromosomal translocation (Philadelphia chromosome) forms a highly active PTK, called the Bcr-Abl oncoprotein [100]. The selective inhibitor to this enzyme designated as STI-571, imatinib, Gleevec, Glivec, or CGP 57148, is now successfully in clinical use. Inhibitors for other enzymes, for example the Raf kinase inhibitor BAY 43-9006 [101] and the PKC inhibitor UCN-01 [102], are the subject of current clinical evaluation for the treatment of cancer.

## CONCLUSIONS

The signal substances of both the nervous and immune systems, namely neurotransmitters and chemokines, have a strong impact on the migration of tumor cells; Fig. (2). A further, recent example for this interaction between the immune system and cancer is delivered by Keith Kelley's



**Fig. (2).** Diagram of the influences of chemokines and neurotransmitters on metastasis development.

group, who showed that the major cytostatic cytokine of the immune system, the tumor necrosis factor, inhibits the insulin-like growth factor-I-induced proliferation of breast carcinoma cells [103]. The resolution of cancer and metastasis development, and the therapeutic inhibition thereof, is therefore a classical example for the necessity for a cooperative, interdisciplinary approach. The neurobiologist will continue to discover and further define new endogenous substances and their corresponding receptors and pathways. The immunologist will in turn determine the role of these substances in immune function, with especial regard to the critical differences (engaged receptors, differential pathways, etc.) between the two systems. Likewise the oncologist and pathologist must identify the specific receptor expression pattern (and pathways involved) of individual tumor cell types, in order to tailor the therapy to each single cancer patient. The pharmacologist has the difficult job of combining this knowledge, that not only gives insight into the complex process of metastasis development and localization, but will illuminate new avenues for a pharmacological inhibition of tumor cell migration as the prerequisite for invasion and metastasis development.

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#### ABBREVIATIONS

ACE	=	Angiotensin-converting enzyme
CGRP	=	Calcitonin gene-related peptide
CCK	=	Cholecystokinin
cAMP	=	Cyclic adenosine-monophosphate
ET	=	Endothelin
FAK	=	Focal adhesion kinase
GABA	=	Gamma-aminobutyric acid
GRK	=	G protein-coupled receptor kinase
GRP	=	Gastrin-releasing peptide
MARCKS	=	Myristoylated alanine-rich C kinase substrate
PLB	=	Phospholamban
PLC	=	Phospholipase C
POMC	=	Proopiomelanocortin
PKA/C	=	Protein kinase A/C
PTK	=	Protein tyrosine kinase
SERCA	=	Sarcoplasmic/endoplasmatic calcium ATPase
SCLC	=	Small cell lung carcinoma
SDF	=	Stromal cell-derived factor
VIP	=	Vasoactive intestinal polypeptide.

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