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# Serotonin and Cancer: What Is the Link?

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# Running title: Serotonin in carcinogenesis

# Abstract:

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic monoamine that acts as a neurotransmitter in the central nervous system, local mediator in the gut and vasoactive agent in the blood. Serotonin exerts its multiple, sometimes opposing, actions through interaction with a multiplicity of receptors coupled to various signalling pathways. In addition to its well-known functions, serotonin has been shown to be a mitogenic factor for a wide range of normal and tumoral cells. Serotonin exhibits a growth stimulatory effect in aggressive cancers and carcinoid more often through 5-HT<sub>1</sub> and 5-HT<sub>2</sub> receptors. In contrast, low doses of serotonin on tumour growth is concentration-dependent. Data are also available on serotonin involvement in cancer cell migration, metastatic processes and as a mediator of angiogenesis. Moreover, the progression of some tumours is accompagnied by a dysregulation of the pattern of serotonin receptor expressions. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma. In some cases, antagonists of serotonin receptors, inhibitors of selective serotonin transporter and of serotonin synthesis have been successfully used to prevent cancer cell growth. This review revaluates serotonin involvement in several types of cancer and at different stages of their progression.

**Key words:** angiogenesis, carcinogenesis, carcinoid, carcinoma, chemotherapy, neuroendocrine cells, serotonin, tumour suppressor

**Contents:** 

- 1. Introduction
- 2. Serotonin receptors and signalling pathways
- 3. Serotonin and carcinogenesis
  - 3.1. Prostate carcinoma
  - 3.1.1. Role of serotonin in prostate carcinoma growth
  - 3.1.2. Serotonin system as a target in prostate carcinoma
  - 3.2. Carcinoma of the urinary bladder
  - 3.3. Small-cell lung carcinoma
  - 3.4. Colorectal carcinoma
  - 3.5. Cholangiocarcinoma
  - 3.6. Choriocarcinoma
  - 3.7. Breast carcinoma
  - 3.8. Hepatocellular carcinoma
  - 3.9. Glioma
  - **3.10. Ovarian tumours**
  - 3.11. Carcinoid tumours
- 4. Serotonin and tumour vasculature
- 5. 5-HT1B as a tumour suppressor
- 6. Conclusions and perspectives

#### 1. Introduction

Serotonin (5-hydroxytryptamine, 5-HT) is a biogenic monoamine that acts as a neurotransmitter in the central nervous system (CNS), local mediator in the gut and vasoactive agent in the blood (this is the origin of the name). Serotonin is synthesized by a two-step pathway from the essential amino-acid tryptophan (Figure 1). Most of the serotonin of the body is located in the periphery. 1) Serotonin is synthesized and stored in the enterochromaffin cells of the intestine. In the gut there are both neural and mucosal sources of serotonin. The enteric neuronal serotonin is clearly required for gastrointestinal motility while mucosal one's has a very minor role [1]. 2) Circulating serotonin is actively taken up and carried primarily in platelets and mast cells that provide a potent reservoir. Serotonin released from platelets is critical for normal wound healing in multiple organs [2].

Within the CNS of vertebrates, a large majority of the cell bodies of serotoninergic neurons are located on or near the midline of the brain stem, in the nine raphe nuclei B1-B9 (B1, raphe pallidus; B2, raphe obscurus; B3, raphe magnus; B4, dorsal to prepositus hypoglossi; B5, raphe pontis; B6, caudal part of raphe dorsalis; B7, raphe dorsalis; B8, centralis; B9, the supralemniscal nucleus). Two subdivisions of the raphe nuclei have further emerged, based on the anatomical connections and physiological characteristics. The neurons of the rostral (B6-B9) and caudal (B1-B5) groups of raphe nuclei give rise to distinct projections, broad and diffuse to the forebrain for the former group and to the hindbrain and spinal cord for the latter one. However, accumulating evidences suggested the existence of a more complex heterogeneity of serotoninergic neurons among the raphe nuclei, with respect to a plethora of characteristics [3].

Serotonin has been linked to a variety of CNS functions such as brain development, circadian rhythm, thermoregulation, cognition, pain, appetite, sexual drive, fear, mood, violent behavior, motor function, neuroendocrine secretion. Moreover, serotonin is implicated in many CNS and psychiatric disorders: Parkinson's disease, depression, hallucination, schizophrenia, bulimia, anxiety, addiction, chronic stress. Consequently, serotoninergic systems are targets for a large array of psychoactive compounds including antidepressants, antipsychotics and hallucinogens [4].

In addition to its well-known functions, serotonin has been shown to be a mitogenic factor for a wide range of normal cells in culture such as vascular smooth muscle cells, lung fibroblasts, renal mesangial cells, hepatocytes, etc... [5-7]. High levels of 5-HT<sub>2A</sub> and 5-HT<sub>2C</sub> receptor expressions have been considered potentially oncogenic in transfected NIH3T3 fibroblasts [8, 9]. Similarly, the expression of 5-HT<sub>2B</sub> receptor correlated with tumorigenicity of a mouse fibroblast cell line and induced tumour formation in nude mice [10]. Serotonin also acts as a growth factor on tumour cells and in some cases, antagonists of 5-HT receptors, inhibitors of SERT and of serotonin synthesis have been successfully used to prevent cancer cell growth [7].

The aim of the present review is to revaluate in light of the recent literature serotonin involvement in several types of cancer, at the different stages of tumour progression and to propose a general view of the link between serotonin and cancer.

## 2. Serotonin receptors and signalling pathways

The classification of serotonin receptors recognizes 14 and 13 receptor subtypes, in rodent and human respectively, spread over seven receptor family [11]. Moreover, several serotonin receptors are coded by complex genes. For example, ten different splice variants of 5-HT<sub>4</sub> receptor are known, termed 5-HT<sub>4</sub> to 5-HT<sub>4</sub> and 5-HT<sub>4</sub>. Serotonin can exert multiple, sometimes opposing, actions, and this is determined by the

characteristics of the receptor with which it interacts and the intracellular signalling pathway coupled to it. With the exception of the 5-HT<sub>3</sub>, all of the serotonin receptors belong to the family of G-protein coupled receptors (Table 1). 5-HT<sub>1A-F</sub>, 5-HT<sub>5A</sub> and <sub>5B</sub> receptors are  $G_i/G_0$  coupled to adenylyl cyclase and downregulate cAMP. 5-HT<sub>2A-C</sub> receptors are  $G_q/G_{11}$  coupled to phospholipase C and upregulate diacylglycerol and inositol triphosphate, resulting in intracellular Ca<sup>2+</sup> release. 5-HT<sub>5B</sub> receptor type is absent in human. 5-HT<sub>4</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> receptors are Gs coupled to adenylyl cyclase and upregulate cAMP [12, 13]. The 5-HT<sub>3</sub> receptor is unique in that it involves a ligand-gated ion channel (Na<sup>+</sup>/K<sup>+</sup>), the binding of serotonin resulting in plasma membrane depolarization. In the CNS, serotonin autoreceptors (5-HT<sub>1A</sub> and 5-HT<sub>1B</sub>) are located on the presynaptic membrane. Binding of serotonin to the autoreceptors acts as a negative feedback against further release of serotonin into the synaptic cleft. In addition to the classic serotonin receptor signalling pathways, there is now increasing evidence that in both normal and transformed cells, many subtypes of serotonin receptors can directly modulate the activity of mitogenic signalling pathways (ERK1/2, Akt) [14, 15].

Table 1.	The Ser	otonin	Receptor	Family
		0.00		

Receptor subtype	Signal transduction mechanism	<b>Agonists</b> <sup>b</sup>	<b>Antagonists<sup>b</sup></b>
1A	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase	BP554 8-OH-DPAT PAPP	NAN-190 Pindobind SDZ21-009 Spiperone
1B	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase	CGS-12066A CGS-12066B CP93129	GR127935 Methiothepin SB216641 SB224289 SDZ21-009
1D	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase	Sumatriptan	GR127935 Metergoline
1E	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase		
1F	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase		
2A	G <sub>q</sub> /G <sub>11</sub> - Phospholipase C	DOI	Ketanserin Mesulergine Metergoline Ritanserin
2B	G <sub>q</sub> /G <sub>11</sub> - Phospholipase C	α-ME-HTP	LY272015 Metergoline SB204741 SB228357 Terguride
2C	Gq/G11 - Phospholipase C	Tegaserod	Mesulergine Metergoline Ritanserin, SB228357 Terguride
3	Ligand-gated cation channel	Phenylbiguanide	Y25130
4	G <sub>s</sub> - Adenylyl cyclase	Cisapride Mosapride	RS-23597-190
5A	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase		
5B <sup>a</sup>	G <sub>i</sub> /G <sub>0</sub> -Adenylyl cyclase		
6	Gs - Adenylyl cyclase		
7	Gs - Adenvlvl cvclase	8-OH-DPAT	Methysergide

<sup>a</sup> : absent in human ; <sup>b</sup> : are listed only the agonists and antagonists used to study the involvement of serotonin in carcinogenesis

# 3. Serotonin and carcinogenesis

Serotonin exhibits a growth stimulatory effect on several types of carcinoma, carcinoids and other tumour cells (Table 2). In contrast, few data are available on serotonin involvement in cancer cell migration and metastatic processes. Serum serotonin level was found to be suitable for prognosis evaluation of urothelial carcinoma in the urinary bladder, adenocarcinoma of the prostate and renal cell carcinoma [16]. In some cases, antagonists of serotonin receptors have been successfully used to inhibit cancer cell growth and the notion of serotonin receptor directed pharmaco-therapy has emerged. Moreover, inhibition of SERT with selective serotonin reuptake inhibitors (SSRI) antidepressant and modulation of serotonin synthesis may represent a therapeutic strategy.

Tuble: Different Studies Renaining a Growth Stimulatory Eneet of Scrotonia on Suncer Sens					
Type of tumour	Cell lines and animal models	<b>Receptors involved</b>	Ref		
Prostate carcinoma	PC3, DU145, LNCaP Xenografted BALB/c mice <sup>a</sup>	5-HT <sub>1A</sub>	[21, 23]		
	PC3, DU145, LNCaP 5-HT <sub>2B</sub> , 5-HT <sub>4</sub>		[24]		
	PC3 5-HT <sub>1A</sub> , 5-HT <sub>1B</sub>		[26]		
	PC3, DU145 5-HT <sub>1A</sub> , 5-HT <sub>1B</sub>		[27]		
Bladder carcinoma	HT1376	5-HT <sub>1A</sub> , 5-HT <sub>1B</sub>	[29]		
Small-cell lung carcinoma	GLC8	5-HT <sub>1A</sub> , 5-HT <sub>1D</sub>	[33-36]		
Colorectal carcinoma	HT29	5-HT <sub>1A</sub> , 5-HT <sub>1B</sub> , 5-HT <sub>3</sub> , 5-HT <sub>4</sub>	[49-51]		
Cholangiocarcinoma	Mz-chA1, HuH28, HUCC-T1, CCLP-1, SG231, TFK-1	5-HT <sub>1A</sub> , 5-HT <sub>2A</sub> , 5-HT <sub>2B</sub> , 5-HT <sub>4</sub> , 5-HT <sub>6</sub>	[53]		
Choriocarcinoma	BeWo, JEG-3	5-HT <sub>2A</sub>	[55]		
Breast carcinoma	MCF-7	5-HT <sub>2A</sub>	[56]		
Hepatocellular carcinoma	Huh7, HepG2, Hep3B Xenografted B/6 mice <sup>a</sup> CCl4-treated mice	5-HT <sub>1B</sub> , 5-HT <sub>2B</sub>	[64, 65]		
Carcinoïd	NCI-H727, NCI-H720, KRJ-I	5-HT <sub>2C</sub>	[81]		
	BON	5-HT <sub>1A</sub> , 5-HT <sub>1B</sub>	[82]		

Table2. Different Studies Relating a Growth Stimulatory Effect of Serotonin on Cancer Cells

<sup>a</sup>: in italic characters, animal models

#### 3.1. Prostate carcinoma

Malignant disease of the prostate gland is the most frequent cancer in males and one of the main causes of death. In the time course of prostatic tumorigenesis, normal prostate progresses to prostatic intraepithelial neoplasia (PIN) and to adenocarcinoma. In aggressive prostate cancers (PC), the tumour cells may metastasize to lymph nodes and bone tissue. Hormone-refractory PC represents the end stage of this progressive and fatal disease. Besides basal and exocrine secretory cells, neuroendocrine (NE) cells represent the third epithelial cell type of the normal prostate gland. NE cells are also present in up to 100 % of prostatic carcinomas, in PIN and in metastatic PC. The NE cells produce and secrete serotonin and a wide range of peptide hormones [17]. It was proposed that NE cells might regulate the growth, differentiation and secretory function of the neighbouring nonneuroendocrine PC cells through paracrine mechanisms [18]. Moreover, NE secretory products may facilitate tumour recurrence by supporting proliferation of neighbouring non-neuroendocrine PC cells [19] and NE differentiation is observed in many metastases. Some studies suggested that the number of NE cells in PC was correlated with stage and Gleason grade, poor prognosis and shortened patient survival.

# 3.1.1. Role of serotonin in prostate carcinoma growth

A link to cancer specific mechanisms of serotonin was provided by its detection in benign prostate epithelium, prostate carcinoma and metastases [20]. Such a link appeared so clear that it was even proposed to

use serum serotonin levels as prognosis marker of adenocarcinoma of the prostate [16]. The action of serotonin on PC is mediated through several receptor subtypes that are present at various tumour stages.  $5-HT_{1A}$ ,  $5-HT_{1B}$ ,  $5-HT_{2B}$  and  $5-HT_4$  receptors were identified in three human prostatic carcinoma cell lines, PC3, DU145 (androgen-unresponsive cells) and LNCaP (androgen-responsive cells) and serotonin was involved in their growth [21-24] (Table 2). A strong expression of  $5-HT_{1A}$  and  $5-HT_{1B}$  receptors was shown in aggressive PC with high Gleason grades (4 and 5) compared to benign prostatic hyperplasia stromal tissue (BPH). The immunostaining of  $5-HT_{1A}$  receptors was also demonstrated in PC cells metastasized to human lymph node and bone and in PC3 cells xenografted subcutaneously in athymic nude mice [23].  $5-HT_{2B}$  receptor expression was associated with pathologically altered prostate as BPH, PIN and also with low and high-grade tumours suggesting that this receptor subtype is involved in the initial stages of prostate tumour.  $5-HT_4$  receptor expression was found predominantly in high-grade tumours suggesting that this receptor subtype may facilitate cell growth in an androgen-depleted environment by a serotonin-induced increase in hormone or growth factor release [24]. Altogether, these results support the hypothesis that NE secretion and serotonin may promote androgen-independence of PC through a paracrine mechanism.

The signal transduction pathways involved in the proliferation of PC cells treated with serotonin was studied in a further study [25]. In PC3, DU145 and LNCaP cell lines the phosphorylation of ERK1/2 and Akt induced by serotonin was reduced by pretreatment with the potent and selective 5-HT<sub>1A</sub> receptor antagonist NAN-190 (Table 1). Moreover, the serotonin-induced cell proliferation and the serotonin induced up regulation of cyclin D1 were reduced after pretreatment of PC3 and DU145 cells with NAN-190, a MEK or a PI3K inhibitor. In LNCaP cells, serotonin-induced ERK1/2 phosphorylation was not related to proliferation but with the acquisition of a NE-phenotype and a concomitant decrease of androgen receptor expression.

In a subsequent work, the role of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors in the proliferation of androgen independent PC3 cells was investigated [26]. Serotonin ( $10^{-8}$  M; 72h) caused a dose dependent PC3 cells proliferation with a maximum of 15% compared to control. Immunostaining and Western blot analysis demonstrated the presence of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors in PC3 cells and in human hormone refractory PC tissue. Western blot also revealed the presence of 5-HT<sub>1D</sub>,  $_{2A}$ ,  $_{2B}$ ,  $_{2C}$  receptor proteins while 5-HT<sub>3</sub>,  $_4$  were not identified. The use of selective antagonists of serotonin receptor subtypes demonstrated that 5-HT<sub>1D</sub>,  $_{2A}$ ,  $_{2B}$ ,  $_{2C}$  receptors are not involved in the regulation of PC3 cells growth. Another study using LNCaP, PC3, DU145 and 22RV1 (an androgen-responding human cell line) cells does not support the hypothesis of a prominent role of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors in the control of PC cell growth at physiological concentration of serotonin.

Quantitative RT PCR demonstrated that 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> mRNA were expressed in the four cell lines used, the expression of the latter being higher than the former. Compared to those of fibroblasts used as reference, the expression is relatively low. PC3 cells showed a dose-dependent stimulatory effect of serotonin on cell growth, with a maximal effect at the supraphysiologic 10<sup>-4</sup> M concentration. In DU145 cells, a slight stimulatory effect was observed only at 10<sup>-4</sup> M [27]. One explanation of the discrepancy between these different studies could be the involvement at high serotonin concentration of other serotonin receptor subtypes that are expressed in PC cells [24].

A correlation was shown between serotonin receptor expression and metastatic PC [23]. Moreover, serotonin promoted invasiveness of PC3 and DU145 cells but not of LNCaP cells. Serotonin-induced cell migration was significantly decreased by the 5-HT<sub>1A</sub> receptor antagonist NAN-190 [25].

# 3.1.2. Serotonin system as a target in prostate carcinoma

The conventional inhibition of androgen-dependent growth of PC cells by androgen ablation therapy represents the primary therapy of locally advanced and metastatic tumours. Unfortunately, approximately 20% of patients are refractory to the androgen ablation and PC cells of responsive patients may lose androgen-sensitivity and become highly invasive and metastatic. Treatment options for androgen independent PC are limited. Several studies have proposed to use antagonists of serotonin receptors as second line treatment for hormone refractory PC (Table 3).

Drug	Target	Effect	Cell lines and animal models	Ref
Pindobind	5-HT <sub>1A</sub>	Inhibition	PC3 (0.5 10 <sup>-6</sup> M) <sup>a</sup> DU145 (0.6 10 <sup>-6</sup> M) <sup>a</sup> LNCaP (> 10 <sup>-4</sup> M) <sup>a</sup> Xenografted BALB/c mice <sup>b</sup>	[21]
NAN-190	5-HT <sub>1A</sub>	Inhibition	PC3 (10 <sup>-6</sup> M) <sup>c</sup> DU145 (10 <sup>-6</sup> M) <sup>c</sup> LNCaP (10 <sup>-6</sup> M) <sup>c</sup>	[23]
SB224289	5-HT <sub>1B</sub>	Inhibition	PC3 (3.23 10 <sup>-6</sup> M) <sup>a</sup>	[26]
SB215505	5-HT <sub>2B</sub>	Inhibition	PC3 (10 <sup>-6</sup> M) <sup>c</sup>	[24]
		None	DU145 LNCaP	
RS-23597-190	$5-\mathrm{HT}_4$	Inhibition	DU145 (10 <sup>-6</sup> M) <sup>c</sup>	[24]
		None	PC3 LNCaP	
Fluoxetine (Prozac <sup>®</sup> )	SERT	Inhibition	PC3 (4 10 <sup>-6</sup> M) <sup>a</sup> DU145 (5 10 <sup>-6</sup> M) <sup>a</sup> LNCaP (6 10 <sup>-6</sup> M) <sup>a</sup> Xenografted BALB/c mice <sup>b</sup>	[22]

 Table 3. Effect of Serotonin Receptor Antagonists and SSRI on the Growth of Prostate Carcinoma Cells

<sup>a</sup>: IC<sub>50</sub> values

<sup>b</sup>: in italic characters, animal models

<sup>c</sup>: concentration at which maximal effect is observed

The results of these studies show that hormone-independent cell lines, DU145 and PC3, are more sensitive to  $5-HT_{1A}$ ,  $5-HT_{2B}$  and  $5-HT_4$  antagonists than androgen-dependent LNCaP cells. The  $5-HT_{1B}$  receptor antagonists NAN-190 and SB224289 induced PC3 cells apoptosis after 24 h and 48 h of incubation, SB224289 causing a greater effect. Thus,  $5-HT_{1B}$  antagonist was proposed to be the more likely therapeutic option for further research [26]. PC cell viability was not affected using NAN-90 in the range between  $10^{-8}$  and  $10^{-4}$  M in the absence of serotonin after 48 h of incubation. In all cell lines, total necrosis was observed after incubation of methiothepin ( $10^{-4}$  M; 12h), a 5-HT<sub>1B</sub> antagonist. NAN-190 ( $10^{-4}$  M) significantly induced apoptotic changes in

PC3 cells only. NAN-90 but not methiothepin induced changes in cell cycle phases, ie  $G_0/G_1$  phase arrest at  $10^{-4}$  M in PC3 and DU145 cells, a possible mechanism in the regulation of cell growth [27]. Recently, a photodynamic therapy using 5-HIAA, the end product of serotonin metabolism, as photosensitizer was investigated for the treatment of prostate and bladder cancers. In this approach, 5-HIAA was selectively activated by irradiation with UVB and 5-HIAA<sup>UVB</sup> was incubated with the androgen-sensitive LNCaP cells, androgen-insensitive PC3 cells and the TCCSUP human urinary bladder carcinoma cells. 5-HIAA<sup>UVB</sup> induced cell death of the three cell types through stress signalling and apoptotic pathways [28].

#### 3.2. Carcinoma of the urinary bladder

Bladder cancer is a malignancy, with a high prevalence and a recurrent nature, arising generally from the urothelium. The most common type is the urothelial cell carcinoma also known as transitional cell carcinoma. The spectrum of clinical severity varies from superficial cancer to invasive cancer into the muscle layer or a metastatic form with poor prognosis. NE differentiation and positive immunohistochemical staining for serotonin were previously reported in such a cancer suggesting the involvement of serotonin in its progression. HT1376, a human high-grade bladder cancer cell line, showed a dose dependent stimulatory effect of serotonin on cell growth, with a maximal 12 % effect ( $10^{-8}$  M, 72 h), compared to the control. Immunostaining and Western blot analysis showed the presence of 5-HT<sub>1A</sub> and 5-HT<sub>1B</sub> receptors in HT1376 cells and in human bladder cancer tissue. Moreover, 5-HT<sub>1B</sub> receptor antagonist (SB224299) and to a lesser extent 5-HT<sub>1A</sub> receptor antagonist (NAN-190) significantly inhibit HT1376 cell growth ( $10^{-4}$  M, 72 h). These results suggest that 5-HT<sub>1B</sub> antagonists could be used in the treatment of bladder cancer [29].

# 3.3. Small-cell lung carcinoma

According to the World Health Organisation, lung NE tumours classification comprises 4 subtypes of tumours: the bronchopulmonary carcinoids (low-grade typical and intermediate-atypical), and the 2 high-grade large-cell NE carcinoma and Small-Cell Lung Carcinoma (SCLC) [30]. SCLC is a very aggressive and early metastatic epithelial tumour associated with tobacco smoking abuse. About 15 % of lung cancers are SCLC and show properties of NE differentiation. The specific cell population that gives rise to SCLC is largely unknown. However, the evidence for the presence of clonal, chemotherapy and radiotherapy-resistant, indefinite self-renewing cancer stem cells in this epithelial tumour is growing [31, 32]. Nicotine stimulates SCLC cell proliferation and is a potent secretagogue for these cells, stimulating the release of substances such as serotonin. Moreover, serotonin stimulates SCLC proliferation in a dose-dependent manner, with a maximal effect at 10<sup>-6</sup> M for the GLC8 cell line. Serotonin stimulation of cell growth is blocked by the 5-HT<sub>1</sub> receptor antagonists.

Therefore, an involvement of a serotoninergic autocrine loop was proposed to control the proliferation of the SCLC, and the 30-50  $10^{-9}$  M serotonin concentration found in the medium after nicotinic stimulation was compatible with this mechanism [33]. 5-HT<sub>1D</sub> receptor was further reported to be at least partly responsible for the mitogenic effect of serotonin in human SCLC cells [34]. A subsequent work indicated that the mitogenic effect of serotonin in SCLC cells also involved 5-HT<sub>1A</sub> receptor for 35 % of serotonin maximal effect. The activation of the two receptor subtypes by selective agonists is additive and account for 100 % of serotonin mitogenic effect in SCLC cells [35]. The mitogenic action of serotonin on human SCLC cells seemed to involve a complex time course interaction between the transduction pathways of the two receptor subtypes [36].

The development of widespread metastases in SCLC is so early that surgical resection of tumours is often not possible. Even if SCLC is highly sensitive to initial cytotoxic chemotherapy and radiotherapy, most patients relapse and die of the disease. Unfortunately, no significant advance was observed in the systemic therapy of SCLC over the past three decades [37, 38]. Early in the nineties, the possibility to inhibit pharmacologically the serotonin autocrine loop was suggested as an interesting therapeutic strategy for the growth control of human SCLC. And indeed, the non-specific 5-HT<sub>1</sub> receptor antagonist methysergide (10<sup>-6</sup> M) completely abolished the mitogenic effect of serotonin. However, the 5-HT<sub>1D</sub> receptor selective agonist sumatriptan stimulated SCLC proliferation with a higher potency than serotonin itself and the selective 5-HT<sub>1D</sub> receptor antagonist GR127935 was much less potent against serotonin effect than against sumatriptan [34, 36]. The 5-HT<sub>1A</sub> receptor antagonist spiperone ( $10^{-5}$ M) completely abolished the mitogenic effect of the 5-HT<sub>1A</sub> receptor agonist 8-OH-DPAT but only partially those of serotonin itself, suggesting an interaction between 5- $HT_{1A}$  and 5- $HT_{1D}$  receptors [35, 36]. More recently, a cell viability assay employing bioluminescence was developed to screen for pharmacological compounds against SCLC. The LOPAC library, which contains 1,280 pharmacological compounds was used in this large-scale screen. Among them, only twelve compounds that target serotoninergic signalling pathways reduced SCLC viability, comprising 5-HT<sub>1B</sub> receptor agonist (CGS-12066A) and antagonist (SB224289), 5-HT<sub>2</sub> receptor antagonist (ritanserin), 5-HT<sub>2B/2C</sub> antagonist (SB228357), 5-HT<sub>1D/2</sub> antagonist (metergoline), 5-HT<sub>1B/1D</sub> antagonist (GR127935) and SSRI (fluoxetine, paroxetine and sertraline). These results corroborated the importance of serotonin in SCLC development and that serotonin signalling may be an interesting chemotherapeutic target [39].

#### 3.4. Colorectal carcinoma

Colorectal cancer is one of the major causes of cancer-related deaths in the world. More than 90% of colorectal carcinomas are adenocarcinomas originating from epithelial cells of the mucosa. Conventional adenocarcinomas are characterized by glandular formation, which is the basis for histologic grading of this tumour [40]. Intraperitoneal injection of  $10 \,\mu g/kg$  serotonin increased the mitotic rate of adenocarcinoma cells in the descending colon of dimethylhydrazine-treated rats [41]. The TPH inhibitor DL-6-fluorotryptophane (6-FT) decreased the tumour cell mitotic rate, whereas the use of non-selective serotoninergic antagonists led to controversial results: 2-bromolysergic acid diethylamide (BOL) decreased, methysergide accelerated and cyproheptadine had no effect on the tumour cell mitotic rate [41, 42]. In a subsequent work, the same laboratory used human colorectal carcinomas growing as xenografts in immunodeficient mice. Treatment of mice with 0.01 or 0.1 mg/kg serotonin failed to accelerate xenograft growth. The failure of treatment with serotonin could be explained by the high doses used or alternatively by the already maximal stimulation of the tumour by the endogenous serotonin. By contrast, 5 mg/kg of the serotoninergic antagonist BW501C retarded tumour growth [43] suggesting that serotonin antagonists may be useful in preventing the repopulation of colorectal carcinomas following antineoplastic therapy [44]. Moreover, citalopram (20 mg/kg) and fluoxetine (10mg/kg) retarded the growth of xenografted colonic tumours and suppressed cell division in dimethylhydrazine-induced colonic tumours in rats [45]. SSRIs might inhibit tumour growth through an antipromoter effect or direct cytotoxic effect and high daily SSRI dose (> 6 x 10<sup>-6</sup> mol per day, during several years) have been further suggested as preventive agent for patient at high risk of developing colorectal cancer [46]. There is evidence that the incidence of depression increases in patients with cancer leading to ongoing debate about whether antidepressant medications may increase or decrease the risk of colon cancer. A recent meta-analysis shows that the use of SSRIs does not increase the risk of colorectal cancer [47]. Interestingly, the used of a population-based cohort of 280 patients suggested that the genetic variations in the SLC6A4 gene which codes for the SERT contributed to poor survival in colorectal cancer patients [48].

More recent studies reconsidered the involvement of serotonin receptor in colorectal cancer and suggested a potential role of 5-HT<sub>1</sub>, 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors in this tumour. Western blot analysis revealed that 5-HT<sub>3</sub> and 5-HT<sub>4</sub> receptors were expressed in HT29 cells and in human colorectal tumour tissues. Serotonin caused a dose dependent proliferation of HT29 cells after 48 h incubation, with a maximum proliferation at a concentration of 12.5  $10^{-6}$  M [49, 50]. Immunohistochemistry of colon cancer tissues from 30 patients in grade II and III colon or rectum adenocarcinoma revealed high membranous staining for 5-HT<sub>1B</sub>, moderate one for 5-

 $HT_{1A}$  in tumour biopsies and very low immunoreactivity in normal colon tissue. Western blot analysis showed high and mild expression of 5- $HT_{1B}$  and 5- $HT_{1A}$  receptors respectively in HT29 cells, compared to normal human colon cells used as negative control [51]. The effects of serotonin receptor agonists and antagonists on HT29 cells growth are presented in Table 4.

Drug	Target	Effect	Ref	
BP554	5 UT	Stimulation (6.25 10 <sup>-6</sup> M) <sup>a</sup>	[51]	
NAN-190	J-111A	Inhibition (10 <sup>-4</sup> M)		
CP93129	5-HT <sub>1B</sub>	Stimulation (6.25 10 <sup>-6</sup> M)	[51]	
SB224289		Inhibition (10 <sup>-4</sup> M)		
Phenylbiguanide	5 ЦТ	Stimulation (6.25 10 <sup>-6</sup> M)	[49, 50]	
Y25130	5-п13	Inhibition (10 <sup>-4</sup> M)	[47, 30]	
Cisapride	5-HT₄	Stimulation (10 <sup>-4</sup> M)	[49, 50]	
RS-23597-190	5 1114	Inhibition (10 <sup>-4</sup> M)	[,00]	

 Table 4. Effect of Serotonin Receptor Agonists and Antagonists on HT29 Cell Growth

<sup>a</sup>: concentration at which maximal effect is observed

The 5-HT<sub>3</sub> receptor antagonist Y25130 had a potent apoptotic and cell cycle arresting effect [49, 50]. On the contrary, no significant effect was found for the 5-HT<sub>4</sub> receptor antagonist RS-23597-190 on cell cycle arrest and cell apoptosis [49, 50]. Cell cycle kinetic analysis revealed an induction of cell cycle arrest at G2/M phase for the 5-HT<sub>1A</sub> receptor antagonist NAN-190 and a much less effect with the 5-HT<sub>1B</sub> receptor antagonist SB224289. Incubation of HT29 cells in the presence of 5-HT<sub>1B</sub> antagonist led to apoptosis while 5-HT<sub>1A</sub> antagonist had low effect [51]. Since it was suggested, that apoptosis was impaired in colon adenocarcinoma, targetting 5-HT<sub>1B</sub> receptor with specific antagonists could be used as an approach to increase the apoptotic process in this cancer. Sulforaphane, an isothiocyanate found in cruciferous vegetables, is a potent chemopreventive agent, with multiple mechanisms of action not fully understood, effective against many carcinogen-induced tumours. In a study which purpose was to identify and characterize novel targets of sulforaphane in human colon adenocarcinoma Caco-2 cells, a potential effect on serotonin-mediated signalling pathways was suggested. In a dose-dependent manner, sulforaphane down-regulated the expression of  $5-HT_{1A}$ ,  $5-HT_{2C}$ ,  $5-HT_3$  receptors and SERT after 48 h of treatment [52].

# 3.5. Cholangiocarcinoma

Cholangiocarcinoma is a biliary tract cancer that can occur in any part of the bile duct, but usually arises in the extrahepatic part of the tube. More of them are adenocarcinomas and these tumours block off the bile ducts. Surgery to completely remove the tumour is the treatment of choice to afford a chance for cure of poor prognosis cholangiocarcinoma. A dysregulation of the cellular machinery responsible for the metabolism of serotonin was observed in human cholangiocarcinoma cell lines, which resulted in an increased production and secretion of the monoamine. Real-time PCR and immunoblotting analysis revealed that TPH1 expression was up-regulated and MAO-A expression decreased in human cholangiocarcinoma cell lines when compared with the H69 non-malignant cholangiocyte cell line. These results were confirmed by immunohistochemical analysis of human liver biopsies of cholangiocarcinoma compared with control samples. Consistent with this finding, an increased production and secretion of serotonin from cholangiocarcinoma was observed in vitro and in vivo. This increased secretion of serotonin may have growth-promoting effects on cholangiocarcinoma cells in vivo. Serotonin treatment (10<sup>-6</sup> to 10<sup>-7</sup> M) significantly increased cholangiocarcinoma proliferation in vitro after 48 h, probably by regulating cell cycle progression, but had no effect on non-malignant cholangiocyte cell line proliferation. Human cholangiocarcinoma cell lines were found to express all serotonin receptor subtypes, the 5-HT<sub>1B</sub>, <sub>1F</sub>, <sub>2B</sub>, <sub>3C</sub> and <sub>7</sub> types being down-regulated and all others up-regulated compared with the H69 human cholangiocyte cell line. Specific inhibition of the 5-HT<sub>1A</sub>, <sub>2A</sub>, <sub>2B</sub>, <sub>4</sub> and <sub>6</sub> receptor types prevented the growthpromoting effects of serotonin on cholangiocarcinoma cell lines. Moreover, in vitro and in vivo inhibition of serotonin synthesis, significantly blocked the growth of cholangiocarcinoma cell lines [53].

## 3.6. Choriocarcinoma

Abnormal growth of trophoblast cells leads to gestational trophoblastic diseases such as the highly malignant choriocarcinoma. 5-HT<sub>2A</sub> receptor receptor was found to be expressed in the human choriocarcinoma cell lines JEG-3 and BeWo as well as in normal human placental tissue. Serotonin had a dose-response stimulatory effect on proliferation of the human placental choriocarcinoma cell lines with a maximal effect at 40

 $10^{-6}$  M of serotonin for JEG-3 cells (25.6 % augmentation) and 20  $10^{-6}$  M for BeWo cells (11.1 % augmentation) compared to untreated control cells [54]. In further studies, BeWo and JEG-3 cells were mainly used as model of human placental trophoblasts to evaluate the potential role of serotonin in placentation and alterations in placental development. In these studies, the authors demonstrated that the mitogenic effect of serotonin shown in choriocarcinoma cells was directly linked to 5-HT<sub>2A</sub> receptor. Pre-treatment of choriocarcinoma cell lines with 10  $\mu$ M ketanserin abolished the increased cell proliferation by serotonin. 5-HT<sub>2A</sub> receptor stimulation by the selective agonist DOI [(±)-2,5-dimethoxy-4-iodoamphetamine hydrochloride)] enhanced cell viability and affected cell cycle progression. The authors also showed that stimulation of the two choriocarcinoma cell lines by DOI activated MEK-ERK1/2 and JAK2-STAT3 signalling pathways [55]. If serotonin constitutes a potent mitogen for neoplastic placental cells, the significance of this mitogenic effect remains to be elucidated.

#### 3.7. Breast carcinoma

Breast cancer is the most common cancer in women worldwide. The predictive value of the actual breast cancer prognosis markers has been of limited benefit so far. Therefore the search of additional parameters is of particular interest for diagnosis and prognosis. A role of serotonin in mammary gland development phenomena was described. Moreover, serotonin is a local regulator of epithelial homeostasis in the normal breast during changes associated with pregnancy, lactation and involution. Dysregulation of epithelial homeostatic systems are associated, in part, with breast cancer initiation and progression. Serotonin promotes the growth of human neoplastic mammary cells, in part through 5-HT<sub>2A</sub> receptors. This was observed for the 5-HT<sub>2A</sub> receptor which is expressed and localized in the plasma membrane of the human breast adenocarcinoma cell line MCF-7. In those cells, serotonin and the selective 5- $HT_{2A}$  receptor agonist DOI stimulated MCF-7 cell growth in a concentration-dependent manner with a maximal effect at  $10^{-5}$  M (52.2 % and 34.4 % augmentation, respectively) [56]. It was shown that extensive alteration of serotonin signalling in mammary gland led to dysregulation of epithelial homeostatic system contributing to breast cancer phenotype. During tumour progression, TPH1 expression increased (corresponding to an increase in serotonin biosynthetic capacity), serotonin receptor pattern became dysregulated and downstream receptor signalling was altered resulting in stimulated proliferation and resistance to apoptose [57]. An immunohistochemical analysis of 102 patients with breast cancer, principally ductal carcinoma, was performed in a tissue microarray to examine the expression of various serotonin receptors. A high expression of 5-HT<sub>1A</sub> was observed in the plasma membrane of breast cancer cells but in contrast strong immunostaining was mainly present in the cytoplasm of non-malignant cells. 5-HT<sub>1B</sub> and 2B expression were highly variable and predominantly observed in the cytoplasm of cancer cells and nonmalignant cells. Strong to moderate expression of 5-HT<sub>4</sub> was found exclusively in the nucleus of malignant and non-malignant breast cells but absent from more than 60% of tumour samples. A significant correlation was found between 5-HT<sub>2B</sub> and estrogen receptor- $\alpha$  and between 5-HT<sub>4</sub> and estrogen- $\alpha$  and progesteron receptors. In contrast, no correlation could be found between any of the receptor subtypes with tumour grade. Further studies are needed to explore the function of serotonin receptor subtypes in breast cancer progression and their potential utility as prognosis markers [58].

The incidence of depression is greatly enhanced in patients with cancer, and the prescription of antidepressant medication for the treatment of this syndrome is a frequent therapeutic option in this sub-population. Studies have suggested that SSRI, when used as an antidepressant medication, promoted breast cancer in animals and women. However, a recent meta-analysis supports the lack of a clinically meaningful association between antidepressant use for more than 1-2 years and the development of breast cancer [59]. On the other side, some studies have suggested that the SSRI fluoxetine may even have anticarcinogenic effect and potential role in the treatment of breast carcinomas [60].

# 3.8. Hepatocellular carcinoma

In adult humans, hepatocellular carcinoma is the most common liver cancer and is a disease with poor prognosis. This malignancy has been increasing, especially in Asian and African populations, and became the third cause of cancer-related death worldwide. Hepatocellular carcinoma is traditionally linked to liver cirrhosis. Alcoholic liver disease and hepatitis B and C are the most common causes of cirrhosis [61]. Rat hepatic serotonin content was directly correlated with the pattern of cell proliferation during liver regeneration and neoplasia. Serotonin content significantly increased in N-nitrosodiethylamine (NDEA) induced hepatocellular carcinoma in rats. In contrast, the number of 5-HT<sub>1A</sub> receptor significantly decreased in NDEA treated rats. These results suggested that hepatic 5-HT<sub>1A</sub> receptor down regulation facilitated hepatocellular carcinoma cell proliferation [62]. During NDEA induced hepatic neoplasia, serotonin content, number and affinity of 5-HT<sub>2C</sub> receptors increased significantly in the rat brainstem and cerebral cortex. Increased plasma norepinephrine levels in NDEA treated rats suggested the involvement of sympathetic stimulation. Thus, 5-HT<sub>2C</sub> receptor upregulation was proposed to facilitate the proliferation of hepatocellular carcinoma cells through sympathetic stimulation [63]. Serotonin promotes cell survival and proliferation of the starved human hepatocellular cancer cell lines Huh7 and HepG2 in a concentration-dependent manner with a maximal effect at  $10^{-4}$  M [64]. In a further study, PCR products were identified for 5-HT<sub>1A</sub>, <sub>1B</sub>, <sub>2B</sub> and <sub>7</sub> in Huh7, HepG2 and Hep3B human hepatocellular cell lines [65]. The use of the specific 5-HT<sub>2B</sub> agonist  $\alpha$ -ME-HTP and antagonist SB204741

allowed to identify this receptor subtype as the mediator of cell survival. In contrast, 5-HT<sub>1A</sub>, <sub>2A</sub> and <sub>7</sub> receptor antagonists had no effect on cell viability. Serotonin mediates cytoprotection in hepatocellular cancer cells after serum deprivation through inhibition of autophagy by activating downstream targets of mTOR. In two murine tumour models, inhibition of 5-HT<sub>2B</sub> signalling decreased hepatocellular cancer cells growth, a proof of principle of in vitro results. These experimental findings were strongly supported by the immunohistochemical analysis of 168 patient biopsies. 5-HT<sub>2B</sub> receptor was significantly associated with human hepatocellular cancer cell growth, as assessed by Ki67 staining [64]. In a subsequent work, using a tissue microarray and immunohistochemistry, serotonin receptor expression was analysed in a total of 176 patients with hepatocellular cancer, and compared on 109 tumour tissues and their corresponding non-tumoral tissues. A similar expression rate was found for 5- $HT_{1A}$  and 5-HT<sub>7</sub> in the two types of tissue. In contrast, 5-HT<sub>1B</sub> and <sub>2B</sub> receptors were significantly overexpressed in tumour tissue compared with the adjacent non-tumoral liver (18 % and 12 % increase, respectively). Among the 176 patients with tumours, 5-HT $_{1B}$  and 5-HT $_{2B}$  were expressed in 32 % and 35 %, respectively, and 12 % were double-positive for the receptors. Both receptors were associated with an increased proliferation index, and  $5-HT_{1B}$  correlated with the size of the tumour, suggesting a role for these two receptors in the biology of hepatocellular cancer. 5-HT<sub>1B</sub> and 5-HT<sub>2B</sub> antagonists, SB216641 and LY272015, respectively, showed a potent cytotoxic effect when tested on the viability and proliferation of Huh7 and HepG2 cell lines [65]. These results suggested that serotonin receptors may represent a potential new therapeutic target in a subset of patients with hepatocellular cancer.

# 3.9. Glioma

Human gliomas are diffuse and often multifocal brain tumours with a high frequency of recurrence. Malignant gliomas are particularly invasive into surrounding brain tissues and render complete surgical resection impossible. Although many therapeutic approaches have been explored, there has been no major improvement over the past 30 years. Even if studies about the effect of serotonin on glioma cells are rare, one work has shown that serotonin stimulates proliferation, migration and invasion of glioma cells *in vitro*. Using eight human glioma cell lines and two normal counterparts, fetal astrocytes from the cerebellum and from the left hemisphere, the expression of seven serotonin receptors was investigated. Among them, the 5-HT<sub>2</sub> receptor was the only to be expressed in all cell lines but weakly in primary culture of rat astrocytes [66]. Five glioma cell lines were growth-stimulated by about 50% when treated with  $10^{-12}$  M serotonin for 48 h. Cell migration and invasion were differentially stimulated by serotonin in the glioma cell lines. Cell migration of two glioma cell lines was stimulated by about 60% at  $10^{-7}$  M serotonin and 88-500 % at  $10^{-6}$  M. With three glioma cell lines,  $10^{-7}$  M

serotonin elicited a 140-230 % increase in invasive cells. Increasing the concentration of serotonin to 10<sup>-6</sup> M induced a 160 % to 13-fold rise in the number of invasive cells in five glioma cell lines. Serotonin seemed to exhibit a stronger invasion-promoting than migration-promoting effect in several glioma cell lines. However, a link was not established between the effect of serotonin on proliferation, migration and invasion of glioma cells and a particular serotonin receptor type [66]. Using rat 9L cells derived from a gliosarcoma, which was chemically induced in CD Fischer rats [67], and a primary culture of rat astrocytes, we investigated the expression of twelve serotonin receptor subtypes by qRT-PCR. We found a different serotonin receptor pattern between rat astrocytes and 9L cells and that the 5-HT<sub>2A</sub> receptor subtype was the only one to be significantly expressed in 9L glioma cells (Figure 2 and supplementary material). Stimulation of rat C6 glioma cells with 10<sup>-5</sup> M serotonin elevated both glial fibrillary acidic protein (GFAP) mRNA and protein contents suggesting that serotonin might induce the differentiation of the cells [68]. Moreover, serotonin increases glial cell line-derived neurotrophic factor (GDNF) release through 5-HT<sub>2</sub> receptors activation in C6 cells [69]. GDNF promotes the survival, activation, proliferation and migration of glioma cell lines suggesting that GDNF release within and around the glioma could be important for malignant tumour formation. It is likely that multiple autocrine signals produced by secreted factors, among which a potential serotonin-stimulated GDNF loop, contribute to invasiveness of aggressive glioma cells [7, 70].

In recent years, the initiation and progression of glioma was shown to be driven by a population of cells with stem cell properties, glioma stem cells, located in niches within tumours [71]. A preleminary study reported an unexpectedly strong inhibition ( $EC_{50} = 0.031 \ 10^{-6} \ M$ ) of mouse neurospheres proliferation, derived from self-renewing multipotent neural stem cells, to the 5-HT<sub>1A</sub> agonist PAPP [72]. A subsequent work showed that sertraline, a SSRI, CGS-12066B, a 5-HT<sub>1B</sub> agonist, and tegaserod, a 5-HT<sub>2C</sub> agonist, killed all glioma neural stem cell lines tested [73]. Future studies using an *in vivo* model are needed to confirm these *in vitro* and unanticipated results.

#### 3. 10. Ovarian tumours

Ovarian cancer is one of the most common cancer among women and is the most lethal gynecological malignancy in developed countries. Among ovarian tumours, epithelial forms represent the largest group. Unfortunately, the five-year survival rate is only 45%, which has not improved over the past three decades. Tumour recurrence and chemoresistance are major causes of poor survival rate of patients, suggesting the existence and involvement of cancer stem cells in ovarian malignancy [74, 75].

An immunohistochemical study of 5-HT<sub>1A</sub>, <sub>1B</sub>, <sub>2B</sub> and <sub>4</sub> receptors in patient tissues revealed a strong expression in benign and non-invasive cancer cells and a decreased expression in invasive malignant cells. Moreover, a correlation was made between a decreased expression of 5-HT<sub>2B</sub> and dissemination of the disease [76]. The present results are in accordance with those on human colon adenocarcinoma which show that serotonin does not influence tumour growth, but strongly reduces cell invasion [77]. These data support a tumour suppressor role for serotonin during the invasive step of ovarian carcinogenesis

#### **3.11. Carcinoid tumours**

Increased serotonin secretion is found in a number of slow-growing NE tumours (NET) of the diffuse NE system often referred to as carcinoid tumours. Carcinoids account for approximately 1 % of all malignancies but their incidence has increased over the last three decades. Carcinoids occur most frequently in the gastrointestinal tract (66 %) and the bronchopulmonary system (31 %) [78]. In cases with development of liver metastases, serotonin reaches the systemic circulation bypassing hepatic inactivation, and engenders the relatively uncommon carcinoid syndrome that is characterized by typical symptoms like cutaneous flushing of the face, neck and chest, secretory diarrhea and heart failure. The carcinoid syndrome is associated with carcinoid tumours of the midgut (small intestine, appendix, caecum, proximal colon). Carcinoid tumours of the foregut (respiratory tract, stomach, duodenum, biliary system, pancreas) produces less serotonin metabolite 5-HIAA is a recommended specific tumour marker for diagnosis and follow-up of carcinoid tumours and carcinoid syndrome [79]. Surgical resection of the tumour and local lymph nodes, possible in 20 % of cases, is the only curative treatment available for carcinoids [78]. Some treatments can palliate symptoms and delay progression, but efficient therapies are needed to regulate carcinoids proliferation.

According to the World Health Organisation, the gastrointestinal tract NET classification distinct the well differentiated NET (benign behaviour, corresponding to the carcinoids), well differentiated NE carcinomas (low-grade malignancy) and poorly differentiated NE carcinoma (small cell with high-grade malignancy) [80]. Lung NET classification comprises four subtypes of tumours: the bronchopulmonary carcinoids (low-grade typical and intermediate-atypical), and the two high-grade large-cell NE carcinoma and small-cell lung carcinoma [30].

The proliferative effect of serotonin in carcinoids was investigated using cell lines of a typical (NCI-H727), an atypical (NCI-H720) bronchopulmonary NET, a small intestinal NET (KRJ-I) and a functioning human pancreatic carcinoid cell line (BON) [81, 82]. Exogenously added serotonin stimulated proliferation in the four human carcinoid cell lines in a concentration-dependent mode. The results of these studies suggested an autocrine proliferation role of serotonin in human carcinoid cells mediated through 5-HT<sub>1A, 1B</sub> receptors in pancreatic tumour and 5-HT<sub>2</sub> receptor in bronchopulmonary NET and small intestinal NET (Figure 3). The effect of Sandostatin LAR®, which was known as an inhibitor of serotonin release and able to inhibit carcinoid growth and hepatic metastases, corroborated the observation that 5-HT receptors regulated tumour growth [81]. Targeting 5-HT receptors could be a complementary strategy in the therapeutic treatment of carcinoids and it was proposed that the 5-HT<sub>2B, 2C</sub> receptor antagonist terguride may have beneficial effects in the treatment of diseases such as carcinoid syndrome in a rat model of long-term hyperserotoninemia [83].

#### 4. Serotonin and tumour vasculature

Serotonin exerts complex actions on blood vessels, dependent on its interaction with a multiplicity of receptors. For example, serotonin-dependent vasoconstriction is caused by activation of 5-HT<sub>1B, 1D</sub> and 5-HT<sub>2A</sub> receptors on vascular smooth muscle cells whereas serotonin-dependent vasodilatation has been linked to activation of 5-HT<sub>2B</sub> receptor subtype present on endothelial cells and the release of relaxing mediators [84]. Several studies have focused on serotonin action on the vascular tone of arterioles feeding the tumours. The hostmodified arterioles feeding a Meth A fibrosarcoma subcutaneously implanted in BALB/c mice have shown high sensitivity to 5-HT<sub>1A,1B</sub> and 5-HT<sub>2A</sub> specific agonists. On the blood vessels feeding the tumour, the serotonin and serotonin receptor agonist vasoconstrictive actions were more potent than on control arterioles and thus could specifically reduce tumour blood flow and interfere with malignant growth [85, 86]. Moreover, serotonin could also inhibit growth of a human colon adenocarcinoma xenotransplanted subcutaneously in athymic nude mice by selectively constricting tumour arterioles [87]. In another study, high dose of serotonin stimulated growth of small cell lung carcinoma grafted in athymic nude mice whereas a low dose inhibited tumour growth. The authors hypothesised that high doses of serotonin may exert a direct mitogenic effect on tumour cells, while low doses reduce tumour growth by acting on the receptors of the tumour vasculature [88]. Several serotonin receptors have been localized on the blood vessels feeding the tumours. 5-HT<sub>1D</sub> and 5-HT<sub>2B</sub> receptors were shown to be highly expressed in vascular endothelial cells in both benign and malignant prostate tissues [23, 24]. Immunohistochemical analysis of patients with breast cancer, principally ductal carcinoma, revealed the expression of 5-HT<sub>1A</sub> and 5-HT<sub>2B</sub> receptors in blood vessel-related cells of malignant and non-malignant specimens [58].

Angiogenesis is an intensive mechanism in tumour tissues that contributes for tumour progression and metastasis, in which neovascular formation supplies oxygen and nutrients to malignant cells. Serotonin, at physiologically relevant concentrations, functions as a potent angiokine (more than VEGF) to promote angiogenesis. Platelet aggregation and blood coagulation are common events in tumour environment. In the thrombotic environment of tumour, platelet aggregation leads to release of serotonin, which may influence angiogenesis, and thus, tumour growth [89]. To establish whether serotonin affected colon carcinoma cell proliferation in vivo, a genetic model of serotonin deficiency outside the CNS was used. Mouse colon carcinoma MC-38 cells were subcutaneously allografted in Tph-/- mice leading to significantly lower tumour volume after 14 days. The serotonin-dependent effect was reproducible when studies were made on allografted Lewis lung carcinoma cell growth. These results implicated serotonin as a key mediator of tumour growth in vivo. However this effect could not be explained by an enhancement of cell proliferation but rather by a regulation of tumour vascularization. Indeed, serotonin was shown to be a positive regulator of angiogenesis in model of allografted tumour growth by suppressing MMP-12 expression in tumour-infiltrating macrophages, thus inhibiting the production of angiostatin, a suppressor of angiogenesis in solid tumours [90]. The effect of a decreased level of serotonin on tumour growth in vivo was also studied using serotonin transporter knockout (5-HTT<sup>-/-</sup>) mice. Lewis lung carcinoma cells, KLN205 lung squamous carcinoma cells or B16F10 melanoma cells were subcutaneously allografted in this genetic model. The tumour growth and the serotonin levels in tumours were markedly attenuated in 5-HTT<sup>-/-</sup> mice compared to allografted 5-HTT<sup>+/+</sup> littermate. These results showed that serotonin levels in the tumour played a crucial role in malignant cell growth, suggesting that physiological levels of serotonin in the tumour are needed for cell growth. Moreover, the effect of serotonin depletion may be attributed to the vasodilatating effect of serotonin on the vessels supplying oxygen and nutrients to tumours. Indeed, specific inhibition of 5-HT<sub>2B</sub> receptor by SB204741 reduced implanted lung cancer tumour growth in mice by suppressing angiogenesis, probably through the inhibition of ERK1/2 and endothelial NOS as was suggested by results obtained in vitro in human umbilical vein endothelial (HUVEC) cells [91]. Using an in vitro model of angiogenesis based on cultured HUVEC cells, the selective 5-HT<sub>4</sub> receptor agonist mosapride citrate was shown to have an antiangiogenic activity which opens the possibility of its application as anticancer agent [92].

#### 5. 5-HT1B as a tumour suppressor

In the last two decades, several studies have linked the human *HTR1B* gene to tumour suppression. Epigenetic alterations have been identified as components of alterations involved in human non-small cell lung cancers (NSCLC). Using two human lung squamous cell carcinoma cell lines, EBC-1 and LK-2, and their normal counterparts, *HTR1B* gene was shown to be aberrantly hypermethylated in its promoter region and transcriptionally silenced in NSCLC. Moreover, decreased mRNA expression of *HTR1B* was observed in 11 of 20 human lung primary cancers. Treatment of LK2 cells with the demethylating agent 5-aza-2'-deoxycytidine induced demethylation and re-expression of *HTR1B* [93]. The analyze of the status of 25 microsatellite markers in 39 cases showed that 6q14.1 is a common deletion region observed in non-Hodgkin lymphomas. 6q14.1 is flanked by the *HTR1B* gene (6q13) and a loss of expression of the gene is observed in non-Hodgkin lymphomas [94]. Moreover, molecular cytogenetic analysis has shown a loss of *HTR1B* gene using a panel of 19 conventional renal cell carcinoma (clear cell) [95]. Indeed, *HTR1B* gene alterations occurred in oral squamous cell carcinomas (OSCC) and they differed significantly between cells from primary tumours and those of OSCC, which had metastasized to neck lymph node [96]. More recently, it was suggested that *HTR1B* loss contributes to osteosarcoma tumorigenesis [97]. Future large-scale studies and follow-up functional experiments are needed to provide a clearer picture of the relevance of *HTR1B* alterations in various human cancers.

#### 6. Conclusions and perspectives

Besides the already known involvement of serotonin receptors in the regulation of several psychiatric and neurological disorders related to serotoninergic neurotransmission, specific receptor subtypes have been associated with solid tumours carcinogenesis. The progression of a solid tumour can be separated into several fundamental stages: 1) growth of the primary tumour; 2) disruption of intercellular junctions, acquisition of motility capacity and invasion; 3) dissemination through interaction with the endothelial barrier of blood or lymphatic vessels (intravasation and extravasation) and finally metastasis (except for brain tumours). Angiogenesis contributes for primary tumour progression but also for metastasis in colonized tissues.

Serotonin acts as a growth factor on several types of tumour cells and it was thus functionally related to oncogenes. However, the effects of serotonin on **tumour growth** are still unclear since opposing effects have been described. For instance, serotonin has been proposed to contribute to cell proliferation in aggressive cancers (prostate carcinoma, bladder cancer, small-cell lung carcinoma, colorectal carcinoma, cholangiocarcinoma, breast carcinoma, hepatocellular carcinoma, glioma, ovarian tumours) and carcinoids. More often the effect of serotonin on cell growth was mediated through  $5-HT_1$  and  $5-HT_2$  receptors and less frequently through  $5-HT_4$  and  $5-HT_6$  receptors. Such observed differences may be related to cell type. In contrast, other studies reported that serotonin can inhibit tumour growth, mainly *via* the vasoconstrictive effects of serotonin on tumour vessels. These results suggest that the role of serotonin on tumour growth is concentration-dependent; when exogenously applied, high doses exert a direct mitogenic effect on tumour cells whereas low doses reduce tumour growth by

decreasing blood supply to the tumour. Moreover, several genetic studies support a tumour suppressor role for the human *HTR1B* gene during lung, renal, oral, osteo carcinogenesis and non-Hodgkin lymphomas.

Few studies have linked serotonin to cancer **cell differentiation**. Stimulation of rat C6 glioma cells with serotonin elevated GFAP mRNA and protein contents suggesting that serotonin might induce the differentiation of these cells [68]. In LNCaP cells, serotonin induced proliferation and was also related with a differentiation and acquisition of NE phenotype [25].

We and others have shown that serotonin can modulate gap junctional intercellular coupling [98]. As gap junctions are involved at several steps of carcinogenesis, the hypothesis of an action of serotonin in cancer process *via* a **modulation of gap junctional intercellular coupling** may be taken into account and needs further investigation [99].

Several data are available on serotonin involvement in **cancer cell migration** and metastatic processes. In rats, an *in vivo* correlation was made between 5-HT<sub>2</sub> receptor and cell lodgement in liver and lung during metastasis. Platelets activated in the presence of tumour cells release serotonin which in turn *via* 5-HT<sub>2</sub> receptor activation played a role in the lodgement of circulating tumour cells [100, 101]. Serotonin promoted the invasiveness of certain PC cells and 5-HT<sub>1A</sub> receptor was shown to be involved in PC cell migration [25]. Serotonin also stimulated migration and invasion of various glioma cell lines [66]. In contrast, other studies supported a tumour suppressor role for serotonin during the invasive step of carcinogenesis. A correlation was made between a decreased expression of 5-HT<sub>2B</sub> and dissemination of ovarian tumour [76]. In human colon adenocarcinoma, serotonin strongly reduced cell invasion [77].

Tumour growth *in vivo* is not only affected by tumour cell proliferation but also by stroma-related factors such as **angiogenesis.** A novel function of serotonin as a mediator of angiogenesis was recently reported *in vivo* [89]. In allografted Lewis lung carcinoma and colon carcinoma cells, the effect of serotonin on tumour growth was explained by a regulation of tumour vascularization [90]. 5-HT<sub>2B</sub> receptor stimulated angiogenesis in allografted Lewis lung carcinoma cells while 5-HT<sub>4</sub> receptor was shown to have an antiangiogenic activity using an *in vitro* model of angiogenesis based on cultured HUVEC cells [91, 92].

In rats, plasma and serum levels of serotonin amounted to about 0.3  $\mu$ M and 15  $\mu$ M, respectively [102]. Are these levels so high to allow serotonin to act on tumour progression? Some of the serotonin effects on cell growth were observed at physiological concentration of serotonin and others at supraphysiological concentrations. One proposed explanation could be the involvement at high serotonin concentration of other serotonin receptor subtypes that are expressed in cancer cells and that have lower affinity for serotonin [24].

Moreover, it was suggested using an *in vivo* model of serotonin transporter knockout (5-*HTT*<sup>-/-</sup>) mice that physiological levels of serotonin in the tumour are needed for cell growth [91].

Data exist for both stimulatory and inhibitory roles of serotonin in carcinogenesis phenomena. These observations suggest that serotonin receptors might react in a tissue-specific manner or that the biological response to serotonin could be determined by the combined effect of several receptors. Moreover, tumour progression seemed to be accompanied by serotonin receptor expression changes. The tightly regulated pattern of serotonin receptors becomes dysregulated in human breast cancer cells, with ectopic expression of some isoforms and suppression of others [57, 58]. The 5-HT<sub>1B</sub>, <sub>1F</sub>, <sub>2B</sub>, <sub>3C</sub> and <sub>7</sub> types were shown to be down-regulated and all the others up-regulated in human cholangiocarcinoma cell lines compared with the H69 human cholangiocyte cell line [53]. 5-HT<sub>1B</sub> and <sub>2B</sub> receptors were significantly overexpressed in tumour tissue compared with the nontumoral adjacent liver [65]. In ovarian cancer, 5-HT<sub>1A</sub>, <sub>1B</sub>, <sub>2B</sub> and <sub>4</sub> receptors were strongly expressed in benign and non-invasive cancer cells of patients and decreased in invasive malignant cells. Moreover, a decreased expression of 5-HT<sub>2B</sub> was linked to the dissemination of the disease [76]. In the same way, we found a different serotonin receptor pattern in rats between rat astrocytes and glioma 9L cells. A systematic analysis of the modulation of serotonin receptor expression during progression of various cancers could be very instructive in order to develop an effective directed pharmaco-therapy.

Serotonin system might represent a target for the prevention and treatment of several cancers. Serotonin receptors, SERT and serotonin synthesis pathways are potential molecular targets in cancer directed pharmacotherapy. The pharmacology of serotonin and serotonin receptors is highly developed and numerous safe and effective serotonin-targeting drugs are available, some of them being currently used in the treatment of CNS disorders. In some cases, antagonists of serotonin receptors have been successfully used to inhibit cancer cell growth and even if these studies were mostly restricted to *in vitro* approaches, the notion of serotonin receptordirected pharmaco-therapy has emerged. Despite the fact that more *in vivo* studies are needed to explore the role of serotonin in cancer growth, we may predict that the use of serotonin receptor agonists and antagonists as anticancer agents in human is limited due to the many side-effects of these compounds. For example, 5-HT2 receptor antagonists induce hematological, nervous, cardiovascular, respiratory and digestive disorders. The selective killing of a population of tumour cells, without affecting normal tissues is a challenge for serotonin receptor-directed pharmaco-therapy. This could be achieved by the development of drug carriers programmed for the selective internalization of serotonin receptor agonists or antagonists exclusively inside a targeted population of tumour cells. The incidence of depression is greatly enhanced in patients with cancer. SSRI antidepressant medications have the general effect of elevating the serotonin levels in the synaptic cleft and plasma by blocking serotonin re-uptake. As a side effect, higher levels of systemic serotonin that result from SSRI antidepressant medication treatment could contribute to the growth of tumours. In contrast, certain SSRIs are cytotoxic on cancer cells *in vitro* at concentrations in the micromolar range. Thus, their applicability as anticancer agents appears limited since concentrations observed in the serum of patients treated with these drugs in standard antidepressant therapeutic regimens are much lower. SSRIs could be interesting chemotherapy agents if their effective concentrations, due to their high lipophilic nature, could be reached locally in cancerous tissues [103]. Despite that strong limitation, strategies using SSRIs against certain cancer cells have been investigated and anticarcinogenic potential of these drugs is emerging [104].

In oncology, serotonin has been already used as tumour marker of gastrointestinal carcinoids, and to some extent of bronchial, hepatic and ovarian carcinoids [16]. Moreover, for neuroendocrine tumours of the pancreatic islet cells and intestinal tract, serotonin could be also used as specific tumour marker [79]. Cancer metastatic form is associated with a poor prognosis. Thus, one of the main thrusts of research in the future is to develop serotonin receptor antagonists and/or SSRIs that could be used in tissue-targeted therapies to prevent progression from superficial disease to invasion and metastatic forms.

**Abbreviations:** 5-HIAA: 5-hydroxyindoleacetic acid; 5-HT: 5-hydroxytryptamine; 5-HTP: 5 hydroxytryptophan; ALDH: aldehyde dehydrogenase, BPH: benign prostatic hyperplasia; CNS: central nervous system; MAO: monoamine oxidase; NDEA: *N*-nitrosodiethylamine; NE: neuroendocrine; NET: neuroendocrine tumour; PC: prostate cancer; PIN: prostatic intraepithelial neoplasia; SERT: selective serotonin transporter; SSRI: selective serotonin reuptake inhibitors; TPH: Tryptophan hydroxylase; VMAT: vesicular monoamine transporter.

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26

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#### **Figure legends**

**Figure 1. Biosynthesis and metabolism of serotonin.** Serotonin is produced in two steps. Tryptophan hydroxylase (TPH) catalyzes tryptophan transformation to 5 hydroxytryptophan (5-HTP). TPH is the ratelimiting enzyme in serotonin synthesis and two isoforms of TPH exist, TPH1 (mostly expressed in the periphery) and TPH2 (the neuron-specific isoform). 5-HTP is further decarboxylated by 5-HTP decarboxylase (or aromatic L-amino acid decarboxylase) to form serotonin. After biosynthesis, serotonin is concentrated in intracellular compartment, the synaptic vesicles in neurons, by the vesicular monoamine transporter (VMAT). Serotonin is metabolized mainly into 5-hydroxyindoleacetic acid (5-HIAA) by monoamine oxidase (essentially MAO-A) and aldehyde dehydrogenase (ALDH) and 5-HIAA is excreted in urine. In the CNS, serotonin is removed from the synaptic cleft by the selective serotonin transporter (SERT) located on the presynaptic neuronal plasma membrane and further either recycled back into presynaptic vesicles or metabolized by MAO within the cytosol.

Figure 2. mRNA expression of serotonin receptors in primary culture of rat astrocytes (black) and in 9L rat glioma cell line (white). Values are expressed as percentage of GAPDH expression (Supplementary material).

**Figure 3.** Serotoninergic autocrine loop involvement in carcinoid tumour proliferation. Exogenously added serotonin stimulated proliferation in a typical (NCI-H727), an atypical (NCI-H720) bronchopulmonary NET and a small intestinal NET (KRJ-I). Treatment of NET cell lines with the -HT<sub>2</sub> receptor antagonist ketanserin and the suicide inhibitor of TPH-1 7-Hydroxytryptophan reversed proliferation through inhibition of 5-HT<sub>2</sub> receptor activation and serotonin synthesis and release, respectively. The carcinoid proliferative role of serotonin is mediated through phosphorylation of ERK1/2 and activation of JNK pathway. BON cell growth (a cell line established from a human pancreatic carcinoid tumour that produces and secretes serotonin) was stimulated by exogenously added serotonin and was inhibited by the 5-HT<sub>1A, 1B</sub> receptor antagonist SDZ 21-009. Moreover, serotonin inhibited cAMP production and SDZ 21-009 completely prevented the reduction of cAMP production evoked by serotonin.



Figure 1



Figure 2



Figure 3

#### SUPPLEMENTARY MATERIAL

#### Includes:

Materials and Methods Table S1 Reference 105

#### 1) Materials and Methods

#### Rat glioma cell line 9L

The permanent, well-characterized rat glioma cell line 9L was a kind gift from Dr. Anne Clavreul (INSERM U646, Angers, France). These cells were maintained in Dulbecco's minimum essential medium (DMEM) high glucose (InVitrogen, Cergy-Pontoise, France) supplemented with 10% FBS, 1% penicillinstreptomycin (Sigma-Aldrich, St Louis, MO) and cultured at 37°C, 5% CO2 in an humidified chamber.

# Rat astrocyte isolation and culture

Primary astrocytes were prepared as described [105] with minor modifications. Cerebral cortices were removed rapidly from three-day-old rats, in DMEM supplemented with 10% FBS, 1% penicillin-streptomycin and 1% glutamine, and the meninges were discarded under stereomicroscopic observation. Cortical tissues were then dissociated by trituration using fire polished Pasteur pipettes in culture medium. Cells in suspension were centrifuged for 10 min at 800xg and resuspended in culture medium. Cells were counted, plated and maintained in culture by renewing the medium (50%) twice a week.

# **Quantitative real-time PCR**

Total RNA was extracted using the RNA Total Isolation Kit (Promega). Reverse-transcription was done with SuperScript II (Invitrogen) as recommended by the manufacturer. Gene expression was assessed through glyceraldehyde-3-phosphate deshydrogenase (GAPDH) expression by quantitative real-time PCR with the GeneAmp 7500 Sequence Detection System and SYBR Green Chemistry (Applied Biosystems). Primer sequences were listed in Table S1. Three independent experiments were done in triplicate.

#### 2) Supplementary table

Table S1: Oligonucleotide sequences for forward and reverse primers.

#### 3) Reference

[105] Nicchia GP, Frigeri A, Liuzzi GM *et al.* Aquaporin-4-containing astrocytes sustain a temperature- and mercury-insensitive swelling in vitro. Glia 2000; 31: 29–38

# Table S1: Oligonucleotide Sequences for Forward and Reverse Primers.

Gene	Assession Number	Primer Sense	Primer Sequence (5' >3')	Amplicon Size	
GAPDH	M17701	Forward	ATTCTACCCACGGCAAGTT	152nh	
	<u>M17701</u>	Reverse	CGCCAGTAGACTCCACGACATA	152p0	
5HT1A	NM 0125851	Forward	GGCAATGCCTGCGTGGTTGC	232nh	
SIIIA	11111_012303.1	Reverse	TGGCGCACAGGTGCAGGATG	232p0	
5HT1R	NM 022225 1	Forward	CTCCCGCCACCTCCCAGACA	201nb	
511110	1001_022223.1	Reverse	GGGTGTGCAGCTTCCGGGTC	20100	
5HT1D	NM 0128521	Forward	GGCCATCACCGATGCCCTGG	126nb	
511110	1111_012052.1	Reverse	GTGGCCTGCCGCCAGAAGAG	12000	
5HT1F	NM 021857.2	Forward	TGGCCTGGCCTTGATGACAACC	81nh	
51111	1001037.2	Reverse	GCTGGGTGGTGCAGCTTCCG	0100	
5нт2а	NM 017254	Forward	TTCACCACAGCCGCTTCAA	109pb	
51112A	1111_017254	Reverse	ATCCTGTAGTCCAAAGACTGGGATT		
5HT2B	NM 017250	Forward	GGCTGATTTGCTGGTTGGATTG	72nh	
511120	1111_017250	Reverse	GGGCCATGTAGCCTCAAACATG	/200	
5HT4A	1120906	Forward	GTGCTAAGGTATACAGTTTTGC	81nh	
51114A	020000	Reverse	CCAGGGACTCTGGGTCATTG	0100	
5HT4R	<u>U20907</u>	Forward	GCTAAGGGATACAGTGGAATGT	77nh	
511140		Reverse	GCAGCCACCAAAGGAGAAGTT	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	
5HT5A	NM_013148.1	Forward	GCCGCTGTGTGTGGTGCTCT	166nb	
51115/1		Reverse	GACGGTGGCGTGACGGACAG	10000	
5HT5B	NM_024395.1	Forward	CACGCGCCACCTGCAGTACA	144nb	
		Reverse	CGCTGCAGCCGAGCATCGTA		
5HT6	L19656.1	Forward	AGCCATGCTGAACGCGCTGT	153nb	
		Reverse	CGCAGCGGCGAGAGGATGAG	15540	
5HT7	L22558.1	Forward	ATTGTGTCCCTGGCGCTGGC	150nb	
51117		Reverse	CGAGGCCGTGCAGCACATGA	10000	