Taylor & Francis
Taylor & Francis Group

REVIEW ARTICLE

OPEN ACCESS Check for updates

Does sympathetic nervous system modulate tumor progression? A narrative review of the literature

Ioannis Stavropoulos[a,b] (iD), Angelos Sarantopoulos[c] and Anastasios Liverezas[d]

aDepartment of Neurophysiology, King's College Hospital, London, UK; bDepartment of Basic and Clinical Neuroscience, King's College London, London, UK; cHematology Division, Department of Internal Medicine, University Hospital of Patra, Patra, Greece; dEuromedica General Hospital of Rhodes, Rhodes, Greece

ABSTRACT

Objective: The role of the sympathetic nervous system (SNS) in tumor development, progression and metastasis is studied for more than half a century and is attracting more attention during the last years. In this narrative review, we aim to a chronological and methodological presentation of the most interesting and pioneering studies on the subject.

Methods: The complexity of the autonomic nervous system's interaction with the immune system, its direct and indirect effects on tumors and their surrounding tissues, plus the diversity and heterogeneity in the design and methodology of the studies, provide hard-to-interpret data and, at times, controversial findings. Studies are categorized into four main groups regarding the distribution of sympathetic nerve fibers inside the tumor, the effect of sympathectomy on cancer progression, the role of neurotransmitters on tumor growth and the impact of sympathetic adrenergic signaling on the anti-tumor immune response.

Results: Studies from all four categories converge to a common point. There is strong evidence that SNS function plays a role in the development and progression of tumors and subsequently the modification of SNS function, locally or diffusely, can affect the course of tumor growth.

Conclusion: The impact of SNS function on cancer behavior may be exerted in two ways, directly via the sympathetic nerve fibers or through widely distributed neurotransmitters. Modification of them, combined or not with treatments altering the immune function, could be the target for future therapeutic implications.

Introduction

It has been more than 40 years since Folkman described that angiogenesis and lymphangiogenesis are essential for tumors' progression and metastasis [1]. The mechanisms that serve these processes have been well studied and now we know that tumor cells trigger angiogenesis and lymphangiogenesis by chemical signals [2,3]. For many years, researchers believed that tumors were not innervated, but data of last decades show that tumors may be able to stimulate the formation of their own innervation in a process named neoneurogenesis. The nervous system seems to interact with the tumor by secretion of factors that mediate tumor progression [4]. Given that several sympathetic neurotransmitters play a role in tumor progression, we reviewed the literature trying to present data about the existence or not of sympathetic innervation within tumors, and the role of the sympathetic nervous system (SNS) in formation and progression of them.

We conducted a literature search on PubMed, EMBASE and Google Scholar databases for original research articles published from January 1949 to April 2020. We used MESH terms and regular keywords for our search. In addition, we used the reference lists of the identified publications to find further relevant articles. Non-full text and non-English studies were excluded.

We attempt to describe studies in chronological order and classify them according to the main methodology, however many studies use multiple methods and thus our classification is not strict (Table 1).

Methods

Studies exploring the presence of sympathetic fibers within tumors

In 1949, Shapiro and Warren published a study exploring the existence of nerve fibers within tumors. They described earlier findings of nerve fibers within tumors, but they had not concluded whether the existence of such fibers was due to neoneurogenesis. In their study, they transplanted Brown-Pearce carcinoma into the eye of 13 rabbits and mice mesothelioma (MT$_8$) in the eye of 22 Guinea pigs. When the

Table 1. Summary of the studies exploring the sympathetic innervation of tumors and the effect of SNS function on tumor growth.

	Type of tumor cells	Method	Main findings
Shapiro 1949	Brown-Pearce carcinoma in rabbit eye and mouse mesothelioma(MT8) in guinea pig eye	Stimulation of transected sympathetic cord with alternate current	Vessel contraction – possible presence of sympathetic fibers within tumor
Stein 1974	Chemically induced chorionepitheliomatous tumor/PC conjugate inoculated subcutaneously in mice	Chemical sympathectomy by injection of 50% ethanol left paravertebral	Higher incidence of tumor growth in sympathectomized vs intact mice
Mattson 1977	Sarcoma or hepatoma transplanted intramuscularly into hindleg of Lister rats	Fluorescence microscopy for visualization of catecholamines	Lack of adrenergic fibers within tumors but existence of them in the surrounding tissue
Borresen 1980	Human bowel adenocarcinomas	Biopsies for determination of nor/-epinephrine and histological examination	Depletion of noradrenaline and lack of nerve fibers within tumor, but presence in the neighboring normal tissue
Grzanna 1985	Murine LPC-1 plasmatocytoma subcutaneous in hindleg or intraperitoneal	Chemical sympathectomy with 6-OHDA, DSP-4, anti-NGF	Inhibition of tumors growth
Chelmicka-Schorr 1985	Clonal lines of C-1300 mouse neuroblastoma	Chemical sympathectomy with 6-OHDA	Inhibition of tumor growth for S-20 clonal line, but not for C-46 and NIE-115 clonal lines
Romeo 1991	Mice breast cancer tumor lines M3 and MM3-LN	Unilateral superior cervical ganglionectomy or sham-operation	Slowing of tumor's growth after sympathectomy
Tatsuta 1992	Wistar rats colon tumors, induced by azoxymethane	Chemical sympathectomy with intraperitoneal 6-OHDA	Reduction in the incidence and number of colon tumors, decreased NE concentration in colon wall
Wang 1994	Juvenile nasopharyngeal angiofibroma	Glyoxylic catecholaminergic histofluorescence method for studying of sympathetic innervations presence	Lack of sympathetic innervation
Yang 2004	Human nasopharyngeal carcinoma tumor cells	NE's effect on expression of VEGF, MMP-2/9	Up-regulation in expression of VEGF, MMP-2/9 and increased invasiveness of all tumor cell lines
Raju 2007	Tongue cancer in dark Agouti male rats	Surgical unilateral and bilateral sympathectomy and sham-surgery	Decreased tumor growth in bilateral sympathectomized rats
Szpunar 2010	Human breast adenocarcinoma cell lines MB-231 and MB231-BR	In vitro: effect of β-AR agonist ISO on VEGF production, In vivo: growth of tumors in mice exposed to chronic stress	ISO increased VEGF production by MB-231BR. Increased tumor growth for both lines and NE concentration by chronic stress
Szpunar 2011	β-AR negative murine mammary adenocarcinoma cell line 4T1	Mice treated with NE reuptake inhibitor desipramine	Increase in tumor NE and transient increase in tumor growth, no effect on VEGF and MMP-9
Lackovicova 2011	BP6-TU2 fibrosarcoma cells injected intraperitoneally in Wistar rats	Chemical sympathectomy by 6-OHDA, add of NE to the cells culture medium	Reduced incidence of tumor. Improved survival after sympathectomy, NE led to elevated proliferation of fibrosarcoma cells
Miyato 2011	Human gastric cancer invading the submucosal or deeper layers	Immunohistochemical staining of TH	Immunoreactivity to TH markedly reduced around arterioles in cancer tissue indicating reduction or loss of SNF
Horii 2012	Implantation of human colon cancer cells to athymic nude mice	Suppression of splenic sympathetic nerve activity by L-carnosine	Inhibition of proliferation of cancer cells
Magnon 2013	PC-3 prostate tumor xenografts in mice, tissue from human radical prostatectomies	Chemical and surgical sympathectomy, pharmacological agonists or antagonists, determination of nerve density	Poor xenografts development after sympathectomy or when stroma cells not expressing β2- and β3-receptors. Lack of adrenergic fibers within tumors
Horvathova 2016	Solid rat intra-abdominal fibrosarcoma, solid murine subcutaneous melanoma, and rat ascites hepatoma	Chemical sympathectomy by 6-OHDA	Attenuated melanoma and fibrosarcoma growth, no effect in incidence and survival of ascites hepatoma
Wrobel 2016	MT/ret mouse model of melanoma	Propranolol administration Flow cytometry	Propranolol treatment delayed primary tumor growth and metastases development. Downregulation of myeloid cell infiltration in tumor microenvironment and promotion of better tumor control by cytotoxic cells.
Buscek 2017	4T1 tumor cells and B16-OVA tumor cells injected subcutaneously in mice	Manipulation of ambient thermal environment, b-blockers and genetic adrenergic receptor knockout mice	CD8+ T cell frequency and functional orientation within the tumor microenvironment regulated by β2- AS in host immune cells. AS and norepinephrine-driven β-AR signaling may alter the immune status of the tumor microenvironment and therefore the use of β-blockers in patients may improve responses to immunotherapy.
Zhang 2017	Hepatoma cell lines HepG2, SMMC-7721, SMMC-7404, PLC, Huh7, MHCC-97H and primary hepatoma cells T127, T420, T421, T1115, T1224 and as408	Immunohistochemical analysis for identification of TH and VAChT. Expression of neurotransmitters' receptors in 12 different hepatoma cells with PCR	Higher expression of both TH and VaChT in HCC than in non-cancer tissue and correlation with disease stage and severity

(continued)

Table 1. Continued.

	Type of tumor cells	Method	Main findings
Nissen 2018	"4242" tumor clone derived from an Em-myc transgenic mouse.	Subcutaneous injection of the nonselective β-AR agonist isoprenaline in mice for investigation of B-cell lymphoma growth and antitumor immunity. Flow cytometry for identification of cellular targets of β-AR signaling	Chronic β-AS eliminated the response to immune therapy and enhanced the growth of transplanted Eμ-myc B lymphoma in mice, inhibited CD8$^+$ T-cell function and suppressed antitumor CD8$^+$ T-cell activity
Bae 2019	Human gastric cancer	Immunohistochemical staining for TH. Immunostaining for β1- and β2-adrenoreceptors	Lower SNS density and β-adrenoreceptor expression associated with worse histological grade
Kamiya 2019	Human breast cancer MDA-MB231-luc and BT549 xenografts in mice, chemically induced breast cancer in Hras128 rats with MNU, human primary breast cancer specimens	Virus-vector-based genetic approach. Immunofluorescence staining	SI of tumors accelerates tumor progression. Stimulation of SN in tumors enhanced breast cancer growth and progression, while stimulation of PN reduced both of them. SN denervation suppresses the expression of immune checkpoint molecules in the tumor microenvironment. Parasympathetic neurostimulation of tumors suppresses the expression of immune checkpoint molecules in the tumor microenvironment. Increased SN and decreased PN density in human breast cancer specimens were associated with poor clinical outcomes and correlated with higher expression of immune checkpoint molecules
Chen 2020	CT26.CL25 colon tumors & 4T1 mammary tumors in BALB/c mice and B16 melanoma cells in C57BL/6 in mice	Physiological, pharmacological and genetic strategies that reduce or block β2-adrenergic signaling to examine the impact of adrenergic stress signaling on the frequency of the radiation-induced abscopal effect.	Pharmacological and physiological blockade of AS improved efficacy of RT and enhanced the abscopal effect (β2-ARs dependent response). Combination of RT and propranolol improved tumor control in 22°C housed mice. Depletion of CD8$^+$ T cells reduced this effect. β-blocker enhanced tumor control and abscopal effect after combined RT and anti-PD-1 antibody.

Abbreviations. PC, Phosphorylcholine; 6-OHDA, 6-hydroxydopamine; DSP-4, N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; Anti-NGF, antibody to nerve growth factor; NE, norepinephrine; VEGF, vascular endothelial growth factor; MMP-2/-9, matrix metalloproteinase-2/-9; ISO, isoproterenol; TH, tyrosine hydroxylase; VAChT, vesicular acetylocholine transporter; HCC, hepatocellular carcinoma cells; PCR, Polymerase chain reaction; SNF, sympathetic nerve fibers; MNU, N-methyl-N-nitrosourea; SI, sympathetic innervation; SN, sympathetic nerve(s); PN, parasympathetic nerve(s); AR: adrenergic receptor(s); AS: adrenergic signaling; RT: radiation therapy.

tumors grew, they stimulated the ipsilateral sympathetic cord and they observed blood vessel contraction, indicating the existence of functioning sympathetic fibers within the tumors. Based on that, they suggested that differences in growth rates of tumors with disturbed innervation could be due to vasomotor changes [5].

On the other hand, two studies published in 1977 and 1980 support that the interior of the tumor lacks sympathetic innervation and this seems to play a role in increased prevalence and progression of tumors [6,7]. Mattson and his colleagues reported that the blood vessels within the tumors lack adrenergic innervation, while blood vessels at the tumor's borders exhibit adrenergic fibers. The authors used the histochemical technique for visualization of catecholamines by fluorescence microscopy. However, they claimed that their results do not preclude the existence of α-adrenergic receptors (α-ARs) at the tumor vessels [6]. In 1980, a Danish research group, knowing that norepinephrine is located in the sympathetic nerve axon terminals, assumed that norepinephrine concentration reflects the density of sympathetic innervation. They studied the presence of norepinephrine within five bowel adenocarcinomas and their neighboring tissue, immediately after surgical removal. Norepinephrine and nerve fibers were absent from tumor

tissue. Norepinephrine concentration was decreased at the borders of the tumor and showed a linear increase with the growing distance from the tumor [7]. Therefore, findings indicated that tumors do not stimulate neoneurogenesis as they do with neoangiogenesis.

In 1994 Wang et al. used the glyoxylic catecholaminergic histofluorescence method to explore the presence of sympathetic innervation in five cases of juvenile nasopharyngeal angiofibroma [8]. In consistence with past studies, they did not find noradrenergic fibers in the interior of the tumors, whilst some sympathetic fibers were present at the tumor's borders and became denser as the distance from tumor increased. The control group of healthy nasal mucosa showed abundant sympathetic innervation.

Miyato et al. also described the reduction or complete absence of sympathetic nerve fibers (SNF) in gastric cancer tissue in 2011. They quantified the distribution of SNF by the use of immunohistochemical staining of tyrosine hydroxylase (TH) in 82 resected samples. Immunoreactivity to TH was markedly reduced around arterioles within cancer tissue and marked loss of SNF was associated with worse clinical outcomes. The reduction of periarteriolar SNF was marked in the central compared to the peripheral areas of the tumor [9]. The authors ascribed the worse outcome to the increased

blood flow within the tumor due to the lack of vasoconstriction effect of SNF.

Bae et al. recently replicated these findings demonstrating a possible protective role of SNS in patients with gastric cancer. The researchers examined the SNF density within and around tumor specimens of 115 patients with gastric cancer. Besides, β1 and β2 adrenergic receptors' (β1- and β2-ARs) expression was assessed by immunohistochemical staining. They evaluated the data for possible associations with histological grade and pathological tumor (pT) and node (pN) stage. Lower SNF density was correlated with higher tumor invasiveness and β1-AR was directly associated with SNS density [10].

Zhang et al. [11] reported opposite findings by examining the distribution of sympathetic and parasympathetic nerve fibers in hepatocellular carcinoma (HCC) cells and their association with disease progression and severity. The investigators used specimens of cancerous tissue from 30 HCC patients. They matched 10 of these specimens with adjacent healthy tissue of the same patients and compared the distribution of autonomic fibers between non-cancerous and cancerous tissue. The density of sympathetic and parasympathetic nerve fibers was again assessed, by TH and vesicular acetylcholine transporter (VAChT) identification respectively, with immunohistochemical analysis. There was a higher expression of both TH and VaChT in HCC compared to non-cancer tissue. Furthermore, TH and VAChT expression were associated with vascular invasion, clinical stage and lower survival. TH intensity was also higher in the recurrence of the disease. The level of expression for various neurotransmitters receptors was examined in twelve types of hepatoma cells. Receptors expressed in most liver tumor cells were β2- and α1- adrenergic, α7 nicotinic AChr and M1 and M3 cholinergic receptors which possibly play a significant role in disease progression [11].

Studies exploring the effect of sympathectomy on tumor growth and progression

Stein et al. in 1974 suggested that sympathetic denervation alters the function of immunological surveillance which leads to attenuation of the tissue's resistance in tumor formation and progression [12]. Following chemical sympathectomy with ethanol in Wistar rats, they induced tumor growth by intraperitoneal injection of chemically induced chorionepitheliomatous tumor cells/phosphorylcholine conjugate. Three weeks later rats were sacrificed. An autopsy showed a much higher incidence of abdominal tumors in sympathectomized rats, which was interpreted as a role of SNS in the initiation of the tumor.

By 1985 a quite distinct perspective about the sympathetic innervation and its effect on tumors appeared. Grzanna et al. performed chemical sympathectomy in mice by two different chemical agents [6-hydroxydopamine (6-OHDA), N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine (DSP-4)] and a nerve growth factor antibody (anti-NGF) that causes immunosympathectomy [13]. All these three agents act with different mechanisms, namely: 6-OHDA acts like a selective neurotoxin for the sympathetic axons, DSP-4 reduces norepinephrine from central and peripheral noradrenergic neurons and treatment of newborn mice with anti-NGF causes immunosympathectomy. LPC-1 murine plasmacytoma cells were injected subcutaneously into the hindleg or intraperitoneally. Sympathectomy resulted in inhibition of the growth of LPC-1 tumors and was similar regardless of the agent used. The common point is that in all three cases the inhibition seems to be related with the destruction of sympathetic axons and not of cells of the adrenal medulla, which should alternate the concentration of circulated norepinephrine. Similarly, researchers did not detect neurochemical markers of sympathetic axons in LPC-1 tumors and thus the inhibition of tumor growth could not be explained by the lack of sympathetic innervation within tumors [13]. The same year Chelmicka-Schorr et al. studied the effect of 6-OHDA-induced sympathectomy on the growth of C-1300 neuroblastoma clonal lines [14]. Chelmicka-Schorr was the first who reported an inhibitory effect of 6-OHDA on tumor growth in 1976 [15], but she mentioned that it does not apply to all kinds of tumors. In 1978, she described that sympathectomy with 6-OHDA suppresses the growth of C-1300 neuroblastoma, and pretreatment of newborn mice with nerve growth factor can augment neuroblastoma growth [16]. However, the following study showed that sympathectomy had a different effect on different clonal lines. S-20 tumors were significantly suppressed in sympathectomized mice while NIE-115 tumors were not influenced and C-46 showed augmented progression in comparison to controls [14]. An explanation of this phenomenon may be the SNS action on tumor growth by secreting a trophic factor influencing C-1300 and its cholinergic clonal line, but no other lines that do not produce neurotransmitters [14]. Similarly, Tatsuta et al. described the inhibition of azoxymethane-induced colon carcinogenesis in Wistar rats that were sympathectomized by repeated injections of 6-OHDA [17]. The incidence of colon tumors was significantly lower in sympathectomized rats than in the control group, indicating that SNS may play a key role in colon carcinogenesis.

Twenty years later, the inhibitory action of 6-OHDA-induced sympathectomy on carcinogenesis was observed in two more studies. Lackovicova et al. reported a decrease in the incidence of BP6-TU2 fibrosarcoma in sympathectomized Wistar rats. In parallel, they described the elevated proliferation of fibrosarcoma cells when they added norepinephrine in their culture. Both findings point out the role of SNS in the proliferation of fibrosarcoma cells [18]. On the same track, Horvathova et al. found significant attenuation of tumor growth in sympathectomized mice injected with B16-F10 melanoma cells or intra-abdominal fibrosarcoma cells. The authors concluded that loss of sympathetic innervation leads to reduced tumor progression in the early stages [19], however, the same group reported that chemical sympathectomy did not influence rat ascites hepatoma progression [20].

On a different approach, Romeo et al. investigated the impact of surgical sympathectomy on the growth of tumors in murine skin [21]. They performed a unilateral superior

cervical ganglionectomy or a sham-operation on syngeneic BALB/c mice, 60–90-days-old. Two weeks later, cells from two breast-cancer tumor lines, were inoculated subcutaneously into the ear of both sympathectomized and sham-operated mice, ipsilateral to the surgical procedure. The tumor lines were M3, which shows high local growth, and MM3-LN that grows at a slower rate but metastasizes earlier to the lung. Tumor's size was assessed every 2–6 days and mice were sacrificed at the 30th (M3) and 38th (MM3-LN) day, after inoculation. Results showed deceleration of growth for M3 tumors in the group with surgical sympathetic denervation of the skin. The same effect was observed on MM3-LN tumors when the first four-time intervals were excluded from statistical analysis (obviously due to the slow growth rate of the cell line). No differences in the number of lung metastasis were seen between mice that underwent superior cervical ganglionectomy and those with sham-operation, for both tumor types. Similarly, 16 years later, Raju et al. reported the effects of surgical sympathectomy on the development of oral cancer [22]. They induced oral cancer in 20 Dark Agouti rats by administration of 4-nitroquinoline-1-oxide in their drinking water. Rats were divided into three groups. The first group was bilaterally sympathectomized, the second one unilaterally sympathectomized and the third one underwent sham surgery. Nineteen weeks later the tumors of the bilaterally sympathectomized group were significantly smaller compared to the sham-operated rats, and the lack of TH immunoreactive nerves suggested the absence of sympathetic fibers within the tumors of bilaterally sympathectomized rats. The data suggest the important role of the sympathetic system in oral cancer progression. The investigators identified 34 genes that were differently expressed in bilateral sympathectomized and sham-operated rats, which suggests that sympathectomy could inhibit tumor growth by altering several genes' expression [23].

Studies exploring the effect of sympathetic neurotransmitters on tumor progression and immune system

Yang et al. used an alternative method to study the effect of sympathetic innervation in nasopharyngeal carcinoma. They examined the impact of the main SNS neurotransmitter, norepinephrine, on the expression of Vascular Endothelial Growth Factor (VEGF) and Matrix Metalloproteinase-2/-9 (MMP-2 and MMP-9) [24]. Data suggest that these proteins have an active role in the formation and progression of tumors [25,26]. Results revealed that norepinephrine caused an increase in the release of these three proteins, which led to an increased invasion of the tumor cells. As it was shown, tumor cells express β1- and β2-ARs, and possibly norepinephrine plays its role by binding to them; this fact could explain the inhibition of norepinephrine action in the release of VEGF, MMP-2 and MMP-9 when researchers blocked the receptors by the administration of the β-blocker propranolol.

Of great interest are the observations regarding the effect of SNS activation on breast cancer progression that was presented by Szpunar et al. at the 17th and 18th Annual Meetings of The Psychoneuroimmunology Research Society [27,28]. The release of norepinephrine and epinephrine due to SNS activation causes an increase in VEGF by stimulation of β-ARs [24,29]. Besides VEGF is a well-known angiogenic factor for many types of cancer cells. The researchers induced an increase in VEGF production in the brain-metastasizing variant of breast carcinoma cell line (MB-231BR) by using the β–AR agonist isoproterenol [27]. Exposure of the mice to chronic stress-induced a growth to MB-231 and MB-231BR tumors and an increase of norepinephrine concentration [27]. The following year, the same group studied the SNS activation and norepinephrine signaling in a tumor cell line that does not express β-ARs (murine mammary adenocarcinoma cell line 4T1). Mice were treated with the norepinephrine reuptake inhibitor desipramine. A transient increase in tumor growth, during the linear phase of tumor growth, was observed. However, levels of tumor VEGF were unchanged [28]. The authors express the dilemma of whether a tumor's response to norepinephrine is dictated by tumor cells versus tumor stroma cells [28]. To locate the site of norepinephrine's action they studied again the metastatic mammary adenocarcinoma cell line 4T1 [30]. They demonstrated that 4T1 tumor cells do not express functioning ARs and thus are unable to respond directly to norepinephrine. Moreover, they observed that mice treated with the norepinephrine reuptake inhibitor desipramine (DMI) or the α2-AR agonist dexmedetomidine (DEX), showed increased tumor growth. They proposed that the ARs of host stromal cells (cells of the immune system, endothelial cells and fibroblasts) could be the site of action of norepinephrine and that their activation could alter the tumor extracellular matrix and regulate tumor collagen structure [30].

Using a distinct approach, the research team of Horii, tried to figure out the connection of the sympathetic activity of spleen and the central immunological regulation affecting tumor progression [31]. Knowing from their experiments that intraduodenal L-carnosine suppresses the sympathetic activity of the spleen they implanted human colon cancer cells in athymic nude mice divided into two groups. The first group was receiving L-carnosine-containing water and the other group water only. The increase of the tumors in L-carnosine group was significantly smaller than the control water group. The possible explanation of the findings was that suppression of splenic sympathetic system leads to increased action of spleen natural killer cells that prevent the proliferation of cancer cells. This study provided important data on the interaction between sympathetic and immune systems that could explain the effect of SNS on carcinogenesis.

A more spherical approach on the topic was published in 2013 by Magnon and his research team [32]. They studied the effect of the autonomic nervous system in the development and dissemination of prostate tumors. They used several methods as chemical and surgical sympathectomy of the prostate gland, pharmacological agonists and antagonists, transgenic mice and prostate cancer tissues from 43 radical prostatectomies. The results indicate that sympathetic nerve outgrowth is essential for the initiation and the early phase of tumor development. The prostate tumor xenografts

showed poor development in the sympathectomized mice and in transgenic mice that did not express β2- and β3-ARs. Microscopic observation of tumor specimens revealed higher nerve density at high-risk than in low-risk adenocarcinomas and more adrenergic fibers were surrounding the tumors than appearing within the tumors. On the contrary, cholinergic fibers infiltrated the tumors. Besides, a higher density of both adrenergic and cholinergic fibers was associated with increased preoperative prostate antigen (PSA) and poor outcome [32]. The described increase in cholinergic fiber density within prostate tumors came to support the cancer-related neurogenesis and axonogenesis in prostate tumors that had already been described a few years before Magnon's study, without however specifying the exact type of fibers found within the tumor [33].

Based on the existing data, De Giorgi investigated the effect of the β-adrenergic antagonist propranolol as a treatment in patients with melanoma and the possibility of increased progression-free survival in these patients. They conducted an open-label study using propranolol as an adjuvant off-label therapy in 19 histologically confirmed stage IB to IIIA cutaneous melanoma patients without the metastatic disease (34 patients were included as a control group). A significant decrease in disease progression within the propranolol treated group (15.8 vs 41.2%) was noted after 3 years of treatment. Overall survival demonstrated a trend to increase in the treated group, but this was not statistically significant, which was attributed to the short follow-up period (3 years) [34].

Studies exploring the effect of sympathetic adrenergic signaling on anti-tumor immune response

In the last few years, several studies provided evidence that adrenergic signaling (AS) modifies the anti-tumor immune response by acting on the ARs of immune cells directly in the tumor microenvironment.

A recent study by Bucsek et al. showed that tumor growth and anti-tumor immune response appear different in tumor-bearing mice housed at 22 and 30 °C and the underlying mechanism is related to β-AS and its inhibitory effect on CD8$^+$ T-cell responses. AS is increased at 22 °C promoting tumor growth and its effect could be reversed by providing the β-blocker propranolol. The effect of propranolol was evident even in tumors from 4T1 tumor cells which lack functional β-ARs. However, the depletion of CD8$^+$ cells eliminated the benefit of propranolol suggesting that the effect of AS targets the immune host cells. Housing temperature had no impact on tumor growth at a model of β2-AR global receptor knockout BALB/c mouse providing additional support that the housing temperature effect on tumor growth is dependent on functional β2-ARs on host cells [35]. Similarly, it has been found that the effect of propranolol on the immunocompetent MT/ret mice model of spontaneous melanoma may alter the anti-tumor immune response. In this immunocompetent model of melanoma, the primary tumor develops in the ocular region and disseminates giving cutaneous and distant metastasis. Propranolol caused a

reduction in myeloid cells (polymorphonuclear neutrophils and macrophages) and enhanced B lymphocytes and CD8$^+$ cells that infiltrate the primary tumor. It delayed the appearance of the primary tumor and cutaneous metastasis and prolonged the progression-free survival. The researchers concluded that they cannot exclude that the effect of propranolol is mediated by the previously observed impact on melanoma cell proliferation and vessel growth [36], however, their observation suggest that propranolol may lead to better tumor immune control [37]. Likewise, Nissen and his group showed that chronic β-AS eliminated the response to immune therapy and enhanced the growth of transplanted Eμ-myc B lymphoma in mice. The findings showed inhibited CD8$^+$ T-cell function and suppressed antitumor CD8$^+$ T-cell activity revealing the inhibitory effect of chronic β-AS on antitumor adaptive immunity [38].

The association between AS and anti-tumor immunity was latterly demonstrated by Chen et al. They used different tumor types (CT26.CL25 & 4T1 in BALB/c mice and B16 melanoma in C57BL/6 mice) and a combination of physiologic, pharmacologic and genetic strategies. The difference in housing temperature led to the different tumor growth rate (increased tumor growth at 22 compared to 30 °C) and similar differences in antitumor responses as previously described. The effect of physiological stress and AS on the outcome of radiation therapy and the abscopal effect was explored. Tumors (CT26.CL25 colon tumor cells or B16 melanoma cells) were implanted in both hind limbs. Radiation therapy was applied only to one limb. Results showed that in mice housed at 22 °C, the blockade of AS by the β-blocker propranolol or by housing at 30 °C improved the efficacy of RT and enhanced the abscopal effect slowing the tumor growth of both the irradiated and contralateral non-irradiated limb. Using β2-AR knock-out mice, they showed that the improved efficacy and abscopal effect is dependent on β2-ARs. The depletion of CD8$^+$ T cells reduced the improved tumor control noticed by a combination of RT and propranolol pointing to the critical role of these cells in the AS mediated immune response. Furthermore, the administration of β-blocker enhanced the tumor control and abscopal effect seen after a combination of RT and checkpoint blockade (anti-PD-1 antibody), suggesting that targeting AS could provide a potential co-immunotherapeutic effect [39].

Kamiya et al. adopted a modern approach and their recently published results provide important new information [40]. The researchers studied the sympathetic and parasympathetic innervation of breast tumors by using models of mice with human breast cancer xenografts and rats with chemically induced breast tumors, as well as mice with spontaneous breast cancer. By utilizing a multi-step retrograde virus-vector-based genetic approach, they were able to manipulate autonomic innervation in a tumor-specific and sympathetic or parasympathetic fiber-type-specific manner and observed the effect on breast cancer progression and the expression of immune checkpoint molecules (PD-1, PD-L1 and FOXP3) which facilitate tumor progression. They found sympathetic innervation within the tumors and showed that activation of tumoral sympathetic nerve fibers

and local release of sympathetic neurotransmitters resulted in increased tumor growth and progression. Daily injection of β-blocker propranolol inhibited the tumor growth of breast cancer in both models when sympathetic nerve fibers were activated, suggesting a β-adrenergic mediated action of sympathetic nerves on tumor growth. On the contrary, genetic sympathetic tumor denervation suppressed tumor growth, distant metastasis forming and expression of immune PD-1, PD-L1 and FOXP3 molecules, while increased the expression of interferon (IFN) on CD4+ and CD8+ tumor-infiltrating lymphocyte (TILs) in the tumor microenvironment of both human breast cancer xenografts in mice and chemically induced breast cancer models. Interestingly, β- and α- adrenergic antagonists were less effective than denervation but propranolol showed enhanced tumor-suppressive effects when bilateral sinoaortic denervation of the baroreflex or restraint stress had preceded. On the other hand, parasympathetic neurostimulation resulted in suppression of tumor growth and decreased expression of the immune checkpoint molecules. Furthermore, the research team examined the autonomic innervation of human breast cancer specimens concluding that a poor clinical outcome was positively related in tumors with higher sympathetic and lower parasympathetic nerve density, respectively. In the same direction tumors from patients with recurrence of the disease showed higher expression of immune checkpoint molecules [40].

Discussion

Tumors are not isolated structures as they interact with the surrounding tissues by cell-to-cell contact and with distal structures via chemical signaling. Recent data indicate that tumors exert, not only neoangiogenesis and lymphangiogenesis, but also neurogenesis, which means that adjacent nerve cells project nerve endings or axons and infiltrate the tumor [4,41]. This process is mediated by neurotrophic factors secreted by tumor cells and indicates a bidirectional effect between the nervous system and cancer cells. Many autonomic neurotransmitters such as norepinephrine, dopamine and substance P have been related to tumor development. Neurotransmitters of the ANS function as mediators and regulators for many tissues and cells of the body. They affect the release of growth and angiogenic factors, regulating the function of cell migration agents as well as cell proliferation and apoptosis. Furthermore, sympathetic postganglionic neurons release norepinephrine and neuropeptide Y, affecting the immune response of T cells, B cells, macrophages and plasma cells. Additionally, they directly affect immune function by innervating the lymphoid organs [42].

Based on the knowledge about the function and interaction of the two super-systems, namely the central nervous and the immune system, we can consider the great influence of SNS, whose postganglionic fibers are distributed throughout the body, but simultaneously its neurotransmitters act via the SNS-adrenal medulla axis, adjusting the "fight or flight" response and the immune system's function [43,44]. This particular dual action of the sympathetic system has been in recent years associated with the effect of stress on the development of cancer [45].

Studies conducted over a period of more than half-century, display important methodological heterogeneity, caused by the rapid technological advances and the evolution in medical knowledge about genetics, neurology and cancer. We could roughly distinguish four main categories of studies.

In the first category, which includes many of the earlier studies, scientists investigated the presence and density of sympathetic fibers within tumors. Findings are particularly important, describing in most cases the absence of sympathetic fibers within tumors and their presence at the limits of the surrounding healthy tissue, with increasing density as we move away from the tumor. The common view that emerges from the majority of the studies describes an absence of sympathetic fibers into the tumor, that further enhances the impression of a central effect of the sympathetic system in tumor growth, by its interaction with cancer cells through messengers, or its impact on overall body's immune response and inflammation. Opposed to this, Shapiro and Warren in 1949 concluded that sympathetic fibers exist within tumors because SNS stimulation caused vasoconstriction to the tumors vessels [5]. Three recent opposing studies support the distribution of sympathetic nerve fibers within human breast cancer xenografts in mice, chemically induced breast tumors in rats, 4T1 tumors developed in the adipose tissue of the rat's breast, but also in human breast cancer specimens and hepatocellular cancerous tissue [11,28,40]. This last finding could reveal a possibility that the existence of sympathetic fibers within tumors differs depending on the type of tumor.

Studies of the second category consider the effect of sympathectomy on tumor growth. The majority of researchers induced chemical sympathectomy by 6-OHDA, while others proceeded to surgical sympathectomy by excision of sympathetic ganglion unilaterally or bilaterally. The two methods of sympathectomy have a major difference. Chemical sympathectomy with 6-OHDA destroys peripheral sympathetic nerve endings, causing a self-oxidation reaction within the cell, but does not destroy the cell bodies of sympathetic neurons. Moreover, the drug does not penetrate the blood-brain barrier thus affecting only peripheral neurons, leading to a generalized peripheral sympathectomy [46]. On the other hand, surgical sympathectomy is localized to the area of distribution of the removed ganglion or the distribution area of transected sympathetic fibers. Despite these differences, sympathectomy in all studies showed an inhibition in the proliferation of tumor cells of all types [12–14,17,18,32], except from two clonal lines of neuroblastoma [14] and rat ascites hepatoma, where chemical sympathectomy with 6-OHDA had no effect [20]; and the study by Stein, where sympathectomy favored tumor growth [12]. Moreover, Raju et al., after comparing the results of unilateral and bilateral surgical sympathectomy in rats with tongue cancer, he revealed that inhibition in cancer growth was induced by bilateral sympathectomy [22]. A possible interpretation of these observations is that sympathectomy affects at a systemic level; therefore the same research team searched and identified

genes associated with carcinogenesis, which were not expressed in rats with bilateral sympathectomy [23].

On the third approach, the effect of SNS neurotransmitters on tumor growth was explored. The results of these studies conclude that the activity of norepinephrine (the main neurotransmitter of SNS), leads to increased tumor cell proliferation *in vitro* and to an augmentation of VEGF and metalloproteinase-2 and -9, which are involved in tumor development [24]. Indeed, the most recent of these studies explored whether this action of norepinephrine is carried through β2- and β3-ARs of cancer cells. In contrast, other investigators have shown that β1-AR density was inversely associated with tumor stage and progression, implying a protective role of the SNS [10]. Several research groups have also suggested that the target of norepinephrine could be the stromal cells of the tumor site and not the tumor itself since norepinephrine did not result in tumor development on genetically engineered laboratory animals that their stromal cells did not express β-ARs [32].

The absence or the reduction of sympathetic fibers within tumors observed in many of the aforementioned studies, and the obvious remark that the SNS function affects tumor development and growth, direct to the hypothesis of a systemic effect of SNS related to the complex interaction mechanisms of the nervous system, immune system and cancer cells through complex networks of chemical messengers. Given that tumorigenesis is a process that involves changes in immunological defense mechanisms of the body, and since there are indications that SNS affects the immune system, plausible hypotheses about the influence of SNS on tumor growth could emerge. There are now impressive anatomic data that reinforce the idea of the central regulation of the immune system via the action of SNS. Sympathetic neurons have connections with primary and secondary lymphoid organs. Additionally, almost all cells of the immune system express ARs, especially the β2- and α-, and their operation is affected by SNS catecholamines, norepinephrine and epinephrine [47]. There is a recent hypothesis that the sympathetic neural way that innervates the lymphoid organs, is distinct and possibly separated functionally from the rest SNS. For example, spleen sympathetic innervation density is three times higher when compared to the kidney and the majority of sympathetic fibers are not associated with normal vasoconstrictor function [48]. Data of many studies about the function of the stress system demonstrate that chronic activation and release of catecholamines and glucocorticoids affect the immune system and can lead to increased tumor growth. Stress alone causes neurochemical changes that promote cell proliferation and subsequently tumorigenesis. The effect appears to be mediated by the ARs and neurogenic inflammation promoted by interleukin-6 (IL-6) [49,50].

Stepping on these observations, the studies of the fourth category provide robust evidence that AS constitutes a way of control of the anti-tumor immune response mainly via β-ARs. The data suggest that increased AS causes a surge in immunosuppressive cells (myeloid-derived suppressor cells and T regulatory cells) with concurrent reduction of cytotoxic immune cells [35,36,38]. Specifically, it is shown that the AS alters the macrophages' fate, by switching the pro-inflammatory M1 population into the immunosuppressive M2 phenotype leading to a reduction of their immune response. Moreover, AS causes limited antigen presentation by dendritic cells followed by impaired cytolytic T cell ability and β2-AR signaling on memory and effector CD8$^+$ T cells diminishes the immune reaction by reducing pro-inflammatory cytokines IL-2 and IFNγ [50]. The action is strongly related to β2-ARs, however, a recent study by Calvani et al. demonstrated the expression of β3-ARs on immune cells and found similar changes in anti-tumor response and a reduction of tumor growth in melanoma when these receptors are blocked [51]. The existing data about the β-AS effect on anti-tumor immune response and several animal studies that have explored the effect of different stress models on tumor growth are summarized in a review paper from Qiao et al. [50]. The results of these studies show that most types of chronic stress (restraint, social isolation, temperature, surgery) promote the tumor growth, however acute stress such as exercise and brief restraint cause decreased tumor growth [50].

In addition, the SNS innervation of bone marrow (BM) seems to play a regulatory role in hematopoiesis and immunological function. Sympathetic neurotransmitters act on ARs expressed on BM niche cells controlling the maintenance of healthy hematopoietic stem cells (HSCs) and their mobilization into peripheral blood [52,53]. BM is commonly affected by metastasis and its dense sympathetic innervation seems to be related to this observation. Increased sympathetic activity facilitated the colonization of circulating tumor cells through an increase in blood vessel density [54]. Nonetheless, the action of SNS on bone marrow is complex and myeloproliferative neoplasms and acute myelogenous leukemia have been related to a degradation of the bone marrow SNS innervation which favors the proliferation of the mutated cells over the HSCs [53,55]. The bulk of evidence demonstrating the effect of SNS and HPA axis activation on immune responses and immune cells is nicely summarized in the recent review of Colon-Echevarria et al. [45].

On the other hand, the systemic regulatory effect of SNS in tumor progression does not exclude the hypothesis of a synchronous regulation by the direct innervation of the tumors. The same applies to the fact that in different studies both unilateral and bilateral sympathectomy resulted in inhibition of tumor growth, implying that the effect of unilateral sympathectomy could be related to the direct tumor innervation or the local effect of the loss of function of peripheral sympathetic fibers. Similarly, chemical sympathectomy did not affect some non-solid tumors, implying that its effect may be applied to solid tumors by loss of local sympathetic input [20]. Moreover, although bilateral sympathectomy was shown to alter gene expression, the levels of circulating plasma catecholamines have not been associated with differential gene expression and there was no correlation between blood and tumor norepinephrine levels in a study on human ovarian cancer [56]. The increased intratumor norepinephrine levels possibly represent sympathetic activation within tumor microenvironment suggesting that the blood-circulated catecholamines may not be the main way of

SNS action on tumor [55]. The suppression of tumor development by genetic sympathetic nerve denervation of the tumor itself supports this local effect of SNS [40]. The use of such techniques will provide new insight into our understanding of local SNS activity on the tumor microenvironment.

The described observations could generate ideas for alternative therapeutic approaches, by targeting AS. Indeed, several studies, both in human populations and experimental animal models, have shown a protective effect of neuromodulators inhibiting AS, such as β-adrenergic antagonists, even though there are some controversies, especially regarding non-solid tumors [55,57,58]. A meta-analysis from Choi et al. showed that the use of β-blockers may improve the length of survival and disease-free survival after treatment in patients with cancer [59]. The effect was stronger on the initial stages of the disease or patients that underwent surgery. In 2018, Yap et al. published a meta-analysis on studies exploring the impact of β-blockers on cancer recurrence and survival. The researchers found no overall effect of β-blockers on recurrence, disease-free survival, or overall survival [60]. Looking at cancer-type-specific studies, the results are still ambiguous. A number of retrospective cohort studies have shown that β-blockers can favorably influence the tumor progression with improved survival and relapse-free survival in patients with breast cancer [61–63], however other studies failed to reveal this effect [64,65]. Furthermore, a study showed that perioperative treatment with propranolol and a COX-2 inhibitor caused a decrease in biomarkers related to breast cancer recurrence and metastasis [66]. Similarly, studies in patients with ovarian cancer have shown a positive effect [67], no significant effect [68,69], or even negative effects [70,71]. In the same manner, evidence has shown that β-blockers may have a role in the treatment of melanoma [72] yet the data are inconclusive and the efficacy not always apparent [73]. The controversies revealed in these studies could be related to the distinct types of cancer, the time of initiation of the treatment with β-blocker (pre- or post-diagnosis), or the type of β-blocker (selective vs nonselective). In addition, the whole observation of the impact of β-blockers in cancer progression is challenged by possible immortal time bias affecting the results of many of the studies [74,75].

Intriguingly, in an animal study, the reduction of AS, either by housing temperature stress relief or by the use of propranolol, appears to bolster the effect of radiation therapy and increase the abscopal responses [39]. In that sense, radiation therapy outcomes could improve by adjuvant β-blocker administration. Undoubtedly, the only way forward is the performance of robust randomized controlled studies in order to clarify the efficacy of b-adrenergic blocking on tumor growth and progression, since the quality of evidence of the available data remains low [58].

Limitations

This is a nonsystematic review of a broad subject and thus there is a possibility of missed studies in the field. Besides, we present and compare findings of studies on animals and human cancerous tissues with significant differences in methodology, leading to an understanding that direct comparisons may not be entirely applicable. Cancer is not a single entity but a widely heterogeneous group of diseases. This suggests that controversial findings may just reflect the effect of SNS in different diseases but more detailed exploration by type of cancer was out of the scope of this review. Finally, we present studies investigating the effect of AS on tumor progression. Even though SNS releases co-transmitters as well, the main focus of this review was on catecholamines; norepinephrine and epinephrine which are the two major neuroeffector molecules.

Conclusion

SNS-tumor interaction is undoubtedly complex. We reviewed a considerable number of articles, covering almost 70 years of research, concerning the sympathetic innervation of tumors and the impact of SNS function on tumor formation and progression. The obtained data are variable but, in all cases, there are indications for an existing interaction. Most, but not all, studies showed that SNF are lacking from tumor parenchyma, but these are abundant in tumor surrounding tissue. AS, central or peripheral mediated, and immune function alterations, diffuse or within the tumor microenvironment, seem to be the two main ways by which SNS interacts with tumors. The findings are fascinating but also conflicting and there is a lot more research to be undertaken until these pathways become well understood. Combined therapeutic interventions targeting both ways may provide new promise in the treatment of cancer.

---

†Professor Kotsiomitis has sadly passed away.

ORCID

Ioannis Stavropoulos http://orcid.org/0000-0002-6920-1826

References

[1] Folkman J. Tumor angiogenesis: therapeutic implications. N Engl J Med. 1971;285(21):1182–1186.

[2] Nishida N, Yano H, Nishida T, et al. Angiogenesis in cancer. Vasc Health Risk Manag. 2006;2(3):213–219.

[3] Eichhorn ME, Kleespies A, Angele MK, et al. Angiogenesis in cancer: molecular mechanisms, clinical impact. Langenbecks Arch Surg. 2007;392(3):371–379.

[4] Mancino M, Ametller E, Gascón P, et al. The neuronal influence on tumor progression. Biochim Biophys Acta. 2011;1816(2):105–118.

[5] Shapiro DM, Warren S. Cancer Innervation. Cancer Res, 1949;9(12):707–711.

[6] Mattsson J, Appelgren L, Hamberger B, et al. Adrenergic innervation of tumour blood vessels. Cancer Lett. 1977;3(0):347–351.

[7] Borresen T, Henriksen OB, Christensen NJ. Decreased norepinephrine concentration in normal tissue neighboring a malignant tumor. Cancer Res. 1980;40(11):4320–4321.

[8] Wang HW, Su WY, Wang JY. Noradrenergic innervation of juvenile nasopharyngeal angiofibroma. Eur Arch Otorhinolaryngol. 1994;251(2):123–126.

[9] Miyato H, Kitayama J, Ishigami H, et al. Loss of sympathetic nerve fibers around intratumoral arterioles reflects malignant potential of gastric cancer. Ann Surg Oncol. 2011;18(8):2281–2288.

[10] Bae GE, Kim HS, Won KY, et al. Lower sympathetic nervous system density and β-adrenoreceptor expression are involved in gastric cancer progression. Anticancer Res. 2019;39(1):231–236.

[11] Zhang L, Wu LLL, Huan HB, et al. Sympathetic and parasympathetic innervation in hepatocellular carcinoma. Neoplasma. 2017;64(6):840–846.

[12] Stein-Werblowsky R. The sympathetic nervous system and cancer. Exp Neurol. 1974;42(1):97–100.

[13] Grzanna R, Frondoza CG, Otten U. Sympathectomy inhibits growth of a murine plasmacytoma tumor. J Auton Nerv Syst. 1985;13(2):149–160.

[14] Chelmicka-Schorr E, Jones KH, Checinski ME, et al. Influence of the sympathetic nervous system on the growth of neuroblastoma in vivo and in vitro. Cancer Res. 1985;45(12):6213–6215.

[15] Chelmicka-Szorc E, Arnason BG. Effect of 6-hydroxydopamine on tumor growth. Cancer Res. 1976;36(7):2382–2384.

[16] Chelmicka-Schorr E, Arnason BG. Modulatory effect of the sympathetic nervous system on neuroblastoma tumor growth. Cancer Res. 1978;38(5):1374–1375.

[17] Tatsuta M, Iishi H, Baba M, et al. Inhibition of azoxymethane-induced experimental colon carcinogenesis in Wistar rats by 6-hydroxydopamine. Int J Cancer. 1992;50(2):298–301.

[18] Lackovicova L, Banovska L, Bundzikova J, et al. Chemical sympathectomy suppresses fibrosarcoma development and improves survival of tumor-bearing rats. Neoplasma. 2011;58(5):424–429.

[19] Horvathova L, Padova A, Tillinger A, et al. Sympathectomy reduces tumor weight and affects expression of tumor-related genes in melanoma tissue in the mouse. Stress. 2016;19(5):528–534.

[20] Horvathova L, Mravec B. Effect of the autonomic nervous system on cancer progression depends on the type of tumor: solid are more affected then ascitic tumors. Endocr Regul. 2016;50(4):215–224.

[21] Romeo HE, Colombo LL, Esquifino AI, et al. Slower growth of tumours in sympathetically denervated murine skin. J Auton Nerv Syst. 1991;32(2):159–164.

[22] Raju B, Haug SR, Ibrahim SO, et al. Sympathectomy decreases size and invasiveness of tongue cancer in rats. Neuroscience. 2007;149(3):715–725.

[23] Raju B, Hultstrom M, Haug SR, et al. Sympathectomy suppresses tumor growth and alters gene-expression profiles in rat tongue cancer. Eur J Oral Sci. 2009;117(4):351–361.

[24] Yang EV, Sood AK, Chen M, et al. Norepinephrine up-regulates the expression of vascular endothelial growth factor, matrix metalloproteinase (MMP)-2, and MMP-9 in nasopharyngeal carcinoma tumor cells. Cancer Res. 2006;66(21):10357–10364.

[25] Sood AK, Bhatty R, Kamat AA, et al. Stress hormone-mediated invasion of ovarian cancer cells. Clin Cancer Res. 2006;12(2):369–375.

[26] Bellon G, Martiny L, Robinet A. Matrix metalloproteinases and matrikines in angiogenesis. Crit Rev Oncol Hematol. 2004;49(3):203–220.

[27] Szpunar MJ, Madden KS, Liverpool KM, et al. Evidence for sympathetic nervous system regulation of breast cancer pathogenesis. Brain, Behav, Immun. 2010;24(1):S40.

[28] Szpunar MJ, Madden KS, Liverpool KM, et al. 157. Sympathetic nervous system innervation and function in a beta-adrenergic receptor negative breast cancer model. Brain, Behav, Immun. 2011;25(2):S224.

[29] Tang J, Li Z, Lu L, et al. β-Adrenergic system, a backstage manipulator regulating tumour progression and drug target in cancer therapy. Semin Cancer Biol. 2013;23(6):533–542.

[30] Szpunar MJ, Burke KA, Dawes RP, et al. The antidepressant desipramine and α2-adrenergic receptor activation promote breast tumor progression in association with altered collagen structure. Cancer Prev Res. 2013;6(12):1262–1272.

[31] Horii Y, Shen J, Fujisaki Y, et al. Effects of L-carnosine on splenic sympathetic nerve activity and tumor proliferation. Neurosci Lett. 2012;510(1):1–5.

[32] Magnon C, Hall SJ, Lin J, et al. Autonomic nerve development contributes to prostate cancer progression. Science. 2013;341(6142):1236361.

[33] Ayala GE, Dai H, Powell M, et al. Cancer-related axonogenesis and neurogenesis in prostate cancer. Clin Cancer Res. 2008;14(23):7593–7603.

[34] De Giorgi V, Grazzini M, Benemei S, et al. Propranolol for off-label treatment of patients with melanoma: results from a cohort study. JAMA Oncol. 2018;4(2):e172908.

[35] Bucsek MJ, Qiao G, MacDonald CR, et al. β-Adrenergic signaling in mice housed at standard temperatures suppresses an effector phenotype in CD8+ T cells and undermines checkpoint inhibitor therapy. Cancer Res. 2017;77(20):5639–5651.

[36] Wrobel LJ, Le Gal FA. Inhibition of human melanoma growth by a non-cardioselective β-blocker. J Invest Dermatol. 2015;135(2):525–531.

[37] Jean Wrobel L, Bod L, Lengagne R, et al. Propranolol induces a favourable shift of anti-tumor immunity in a murine spontaneous model of melanoma. Oncotarget. 2016;7(47):77825–77837.

[38] Nissen MD, Sloan EK, Mattarollo SR. β-Adrenergic signaling impairs antitumor CD8+ T-cell responses to B-cell lymphoma immunotherapy. Cancer Immunol Res. 2018;6(1):98–109.

[39] Chen M, Qiao G, Hylander BL, et al. Adrenergic stress constrains the development of anti-tumor immunity and abscopal responses following local radiation. Nat Commun. 2020;11(1):1821.

[40] Kamiya A, Hayama Y, Kato S, et al. Genetic manipulation of autonomic nerve fiber innervation and activity and its effect on breast cancer progression. Nat Neurosci. 2019;22(8):1289–1305.

[41] Ondicova K, Mravec B. Role of nervous system in cancer aetiopathogenesis. Lancet Oncol. 2010;11(6):596–601.

[42] Raju B, Ibrahim SO. Pathophysiology of oral cancer in experimental animal models: a review with focus on the role of sympathetic nerves. J Oral Pathol Med. 2011;40(1):1–9.

[43] Elenkov IJ, Wilder RL, Chrousos GP, et al. The sympathetic nerve–an integrative interface between two supersystems: the brain and the immune system. Pharmacol Rev. 2000;52(4):595–638.

[44] Kenney MJ, Ganta CK. Autonomic nervous system and immune system interactions. Compr Physiol. 2014;4(3):1177–1200.

[45] Colon-Echevarria CB, Lamboy-Caraballo R, Aquino-Acevedo AN, et al. Neuroendocrine regulation of tumor-associated immune cells. Front Oncol. 2019;9(1077):1077.

[46] Moynihan J, Kruszewska B, Madden K, et al. Sympathetic nervous system regulation of immunity. J Neuroimmunol. 2004;147(1–2): 87–90.

[47] Bellinger DL, Lorton D. Autonomic regulation of cellular immune function. Auton Neurosci. 2014;182:15–41.

[48] Janig W. Sympathetic nervous system and inflammation: a conceptual view. Auton Neurosci. 2014;182:4–14.

[49] Saloman JL, Albers KM, Rhim AD, et al. Can stopping nerves, stop cancer? Trends Neurosci. 2016;39(12):880–889.

[50] Qiao G, Chen M, Bucsek MJ, et al. Adrenergic signaling: a targetable checkpoint limiting development of the antitumor immune response. Front Immunol. 2018;9:164.

[51] Calvani M, Bruno G, Dal Monte M, et al. β3 -Adrenoceptor as a potential immuno-suppressor agent in melanoma. Br J Pharmacol. 2019;176(14):2509–2524.

[52] Hanoun M, Maryanovich M, Arnal-Estape A, et al. Neural regulation of hematopoiesis, inflammation, and cancer. Neuron. 2015; 86(2):360–373.

[53] Laplane L, Duluc D, Bikfalvi A, et al. Beyond the tumour microenvironment. Int J Cancer. 2019;145(10):2611–2618.

[54] Hanns P, Paczulla AM, Medinger M, et al. Stress and catecholamines modulate the bone marrow microenvironment to promote tumorigenesis. Cell Stress. 2019;3(7):221–235.

[55] Cole SW, Nagaraja AS, Lutgendorf SK, et al. Sympathetic nervous system regulation of the tumour microenvironment. Nat Rev Cancer. 2015;15(9):563–572.

[56] Lutgendorf SK, DeGeest K, Sung CY, et al. Depression, social support, and beta-adrenergic transcription control in human ovarian cancer. Brain Behav Immun. 2009;23(2):176–183.

[57] Armaiz-Pena GN, Cole SW, Lutgendorf SK, et al. Neuroendocrine influences on cancer progression. Brain Behav Immun. 2013; 30(25):21.

[58] Fumagalli C, Maurizi N, Marchionni N, et al. β-blockers: their new life from hypertension to cancer and migraine. Pharmacol Res. 2020;151:104587.

[59] Choi CH, Song T, Kim TH, et al. Meta-analysis of the effects of beta blocker on survival time in cancer patients. J Cancer Res Clin Oncol. 2014;140(7):1179–1188.

[60] Yap A, Lopez-Olivo MA, Dubowitz J, et al. Effect of beta-blockers on cancer recurrence and survival: a meta-analysis of epidemiological and perioperative studies. Br J Anaesth. 2018;121(1):45–57.

[61] Barron TI, Connolly RM, Sharp L, et al. Beta blockers and breast cancer mortality: a population- based study. J Clin Oncol. 2011; 29(19):2635–2644.

[62] Melhem-Bertrandt A, Chavez-Macgregor M, Lei X, et al. Beta-blocker use is associated with improved relapse-free survival in patients with triple-negative breast cancer. J Clin Oncol. 2011; 29(19):2645–2652.

[63] Powe DG, Voss MJ, Zanker KS, et al. Beta-blocker drug therapy reduces secondary cancer formation in breast cancer and improves cancer specific survival. Oncotarget. 2010;1(7):628–638.

[64] Sorensen GV, Ganz PA, Cole SW, et al. Use of β-blockers, angiotensin-converting enzyme inhibitors, angiotensin II receptor blockers, and risk of breast cancer recurrence: a Danish nationwide prospective cohort study. J Clin Oncol. 2013;31(18): 2265–2272.

[65] Cardwell CR, Coleman HG, Murray LJ, et al. Beta-blocker usage and breast cancer survival: a nested case-control study within a UK clinical practice research datalink cohort. Int J Epidemiol. 2013;42(6):1852–1861.

[66] Shaashua L, Shabat-Simon M, Haldar R, et al. Perioperative COX-2 and β-adrenergic blockade improves metastatic biomarkers in breast cancer patients in a phase-II randomized trial. Clin Cancer Res. 2017;23(16):4651–4661.

[67] Baek MH, Kim DY, Kim SO, et al. Impact of beta blockers on survival outcomes in ovarian cancer: a nationwide population-based cohort study. J Gynecol Oncol. 2018;29(6):e82.

[68] Cho MA, Jeong SY, Sohn I, et al. Impact of angiotensin receptor blockers, beta blockers, calcium channel blockers and thiazide diuretics on survival of ovarian cancer patients. Cancer Res Treat. 2020;52(2):645–654.

[69] Majidi A, Na R, Dixon-Suen S, et al. Common medications and survival in women with ovarian cancer: a systematic review and meta-analysis. Gynecol Oncol. 2020;157(3):678–685.

[70] Gonzalez R, Gockley AA, Melamed A, et al. Multivariable analysis of association of beta-blocker use and survival in advanced ovarian cancer. Gynecol Oncol. 2020;157(3):700–705.

[71] Couttenier A, Lacroix O, Silversmit G, et al. Beta-blocker use and mortality following ovarian cancer diagnosis: a population-based study. Cancer Epidemiol. 2019;62:101579.

[72] De Giorgi V, Geppetti P, Lupi C, et al. The role of β-blockers in melanoma. J Neuroimmune Pharmacol. 2020;15(1):17–26.

[73] McCourt C, Coleman HG, Murray LJ, et al. Beta-blocker usage after malignant melanoma diagnosis and survival: a population-based nested case-control study. Br J Dermatol. 2014;170(4): 930–938.

[74] Weberpals J, Jansen L, Carr PR, et al. Beta blockers and cancer prognosis – the role of immortal time bias: a systematic review and meta-analysis. Cancer Treat Rev. 2016;47:1–11.

[75] Weberpals J, Jansen L, van Herk-Sukel MPP, et al. Immortal time bias in pharmacoepidemiological studies on cancer patient survival: empirical illustration for beta-blocker use in four cancers with different prognosis. Eur J Epidemiol. 2017;32(11):1019–1031.