Review

Cancer Research

Nerve–Cancer Cell Cross-talk: A Novel Promoter of Tumor Progression

Phillip Jobling[1,2], Jay Pundavela[1,2], Sonia M.R. Oliveira[1,2], Séverine Roselli[1,2], Marjorie M. Walker[2,3], and Hubert Hondermarck[1,2]

Abstract

Recent studies have revealed the essential role played by nerves in tumor progression. Nerves have been shown to infiltrate the tumor microenvironment and actively stimulate cancer cell growth and dissemination. This mechanism involves the release of neurotransmitters, such as catecholamines and acetylcholine, directly into the vicinity of cancer and stromal cells to activate corresponding membrane receptors. Conversely, the secretion of neurotrophic growth factors by cancer cells drives the outgrowth of nerves in solid tumors. This reciprocal interaction between nerves and cancer cells provides new insights into the cellular and molecular bases of tumorigenesis and points to the potential utility of antineurogenic therapies. This review will discuss our evolving understanding of the cross-talk between nerves and cancer cells. *Cancer Res; 75(9); 1777–81. ©2015 AACR.*

Introduction

The peripheral nervous system can be viewed as a neuronal circuit that connects all body parts and organs to the central nervous system and thus the brain. Sensory and motor nerves are mediators of environmental adaptation to the outside world through cognitive integration and muscular movement. Interestingly, the same concept applies to internal organs via autonomic nerves. Sympathetic and parasympathetic nerves reach most internal organs and orchestrate tissue homeostasis through direct innervation and release of neurotransmitters such as catecholamines and acetylcholine. Despite early studies dating back from the 1940s and showing an impact of denervation in cancer (1–3), the role of nerves in cancer initiation and progression has remained unclear. However, recent evidence has led to the crystallization of a new paradigm: that the infiltration of the tumor microenvironment by nerves, termed tumor neoneurogenesis or axonogenesis, plays an active role in cancer progression. Infiltrating nerve fibers stimulate tumor growth and dissemination, and reciprocally tumor cells drive nerve outgrowth in a cross-talk that contributes to tumor progression. This review aims to outline the latest developments about the nerve–cancer cell interaction and to explore the potential value of antineurogenic therapies.

[1]School of Biomedical Sciences and Pharmacy, University of Newcastle, Callaghan New South Wales, Australia. [2]Hunter Medical Research Institute, University of Newcastle, Callaghan New South Wales, Australia. [3]School of Medicine and Public Health, Faculty of Health and Medicine, University of Newcastle, Callaghan New South Wales, Australia.

Corresponding Author: Hubert Hondermarck, School of Biomedical Sciences and Pharmacy, Life Science Building, University of Newcastle, University Drive, Callaghan NSW 2308, Australia. Phone: 61-492-18830; Fax: 61-2492-18831; E-mail: hubert.hondermarck@newcastle.edu.au

doi: 10.1158/0008-5472.CAN-14-3180

Nerves in the Tumor Microenvironment and Their Impact on Cancer Progression

The tumor microenvironment is crucial to cancer progression. The interaction of cancer cells with the stroma, including endothelial cells, immune cells, fibroblasts, and the extracellular matrix, has been established, and this has led to innovative anticancer therapies, for example, those targeting angiogenesis. In contrast, until recently, the presence and role of nerves in the tumor microenvironment have received little attention. It is established that cancer cells can grow around existing nerves and eventually invade them, in a process called perineural invasion (4). This is generally associated with a poor prognosis and can cause severe pain as demonstrated in pancreatic cancer (5). However, during perineural invasion, nerves are passive, in that they essentially provide a route for cancer cell dissemination. The paradigm change established very recently is that the converse phenomenon, that is, the infiltration of tumors by growing nerves (tumor neoneurogenesis or axonogenesis), has been evidenced and linked to cancer progression.

In a landmark paper, Magnon and colleagues (6) have demonstrated that autonomic nerve sprouting in prostate tumors is essential to prostate cancer progression. Sympathetic and parasympathetic nerves were found to be necessary throughout all phases of prostate cancer development in the mouse. On one hand, the early phases of tumor development were found preventable by sympathectomy or genetic deletion of β-adrenergic receptors, and on the other hand, tumors were also infiltrated by parasympathetic cholinergic fibers that promoted cancer dissemination. Catecholamines and acetylcholine, secreted by sympathetic and parasympathetic nerves, were responsible for the stimulation of prostate tumor growth and metastasis, respectively. Although actions of the sympathetic and parasympathetic nervous systems are classically in opposition, this study suggests that in cancer, they are in fact complementary, where sympathetic nerves stimulate early phases and parasympathetic nerves activate the late metastatic process. Interestingly, stromal cells were found to express β-adrenergic receptors and muscarinic receptors, and to

be targeted by the corresponding ligands, catecholamines, and acetylcholine, secreted by nerves. This reinforces the concept that stromal cells interact with tumor cells and impact their biology. In human prostate tumors, the density of nerves was directly correlated to the Gleason prostate cancer score and tumor aggressiveness, thus providing a clinical relevance to the findings in mice. Taken together, this study was the first to clearly demonstrate that the nervous system is involved in cancer progression and that autonomic nerves are an essential component of the tumor microenvironment, participating in cancer growth and metastasis.

Furthermore, a recent study by Zhao and colleagues (7) has shown that denervation suppresses gastric tumorigenesis. Using a mouse model of gastric cancer, surgical and pharmacologic denervation of the stomach, by vagotomy or local injection of neurotoxic agents, strongly reduced tumor incidence and progression. In addition, denervation was able to enhance the therapeutic effect of systemic chemotherapy and may therefore be a feasible strategy for the control of gastric cancer. In terms of mechanisms, the denervation-induced suppression of gastric tumorigenesis was associated with the inhibition of Wnt signaling and subsequent suppression of stem cell expansion mediated through cholinergic signaling. Cholinergic nerves have been shown to regulate murine gastrointestinal epithelial proliferation (8), and therefore the trophic function of cholinergic nerves for normal gastric epithelial cells appears to extend to the corresponding cancer cells, thus contributing to gastric cancer progression.

Interestingly, it has already been shown that receptors for autonomic neurotransmitters can stimulate cancer cell growth through the activation of corresponding signaling pathways. For instance, the activation of β-adrenergic receptors is essential to malignant growth in ovarian cancer (9) and accelerates pancreatic (10) and pulmonary (11) cancer cell growth and invasion. In addition to their effect on cancer cells, autonomic neurotransmitters are also known to stimulate endothelial cells, immune cells, and fibroblasts (12) and therefore their impact on the tumor microenvironment is likely to be broader and extend beyond a direct stimulation of cancer cells. Lymphocytes express catecholamine receptors and macrophages respond to acetylcholine and in many cases, these interactions are immunosuppressive and anti-inflammatory (13). However, until now the clinical relevance of autonomic neurotransmitters in cancer was unclear as their concentration in the blood is not sufficient to induce a potent effect on cancer cell growth. The discovery of the impact of nerves in prostate and gastric tumors sheds a new light on the role of autonomic neurotransmitters on tumor cell growth, as nerve fibers can release these neurotransmitters directly into the vicinity of cancer cells to stimulate their survival, proliferation, and ability to spread.

Neurogenic Activity of Tumor Cells: The Role of Neurotrophic Growth Factors

Although the two pioneer studies in prostate and gastric cancer revealed the impact of nerves in cancer progression (6, 7), the key drivers of neuron outgrowth in tumors were not identified. However, two recently published studies in prostate cancer have shown that the attraction of nerve fibers is mediated through the production of neurotrophic growth factors by cancer cells. Pundavela and colleagues (14) have shown that proNGF, the precursor of nerve growth factor (NGF), is expressed in prostate cancer cells and a key driver of nerve infiltration. ProNGF was found to be overexpressed in prostate cancer cells compared with normal and benign hyperplastic prostate epithelial cells. ProNGF was correlated to tumor aggressiveness and low-risk tumors (Gleason score = 6) contained significantly less proNGF than intermediate and high-risk tumors (Gleason score $\geq$7). Interestingly, although at this stage, there are no supporting mechanistic animal studies, prostate cancer cells *in vitro* were found able to stimulate neuron outgrowth through the secretion of proNGF (14). Whether or not proNGF acts directly on neurons, or is first processed into NGF, is still to be determined, but this study showed that proNGF/NGFs are involved in the neurogenic ability of prostate cancer cells. ProNGF/NGFs have been shown to be involved in perineural invasion (5), and the study by Pundavela and colleagues (14) indicates that these growth factors also participate in tumor neoneurogenesis. Another investigation by Dobrenis and colleagues (15) has shown that the hematopoietic growth factor G-CSF constrains the growth of prostate cancer cells by supporting the survival of sympathetic nerve fibers. Growth factors generally have pleiotropic functions and although G-CSF was not originally described as a neurotrophic molecule, this study shows that it contributes to neoneurogenesis in prostate cancer. Together the studies by Pundavela and colleagues (14) and Dobrenis and colleagues (15) demonstrate that prostate tumors have the ability to attract nerve fibers through the production and release of neurotrophic growth factors. In addition, cancer cells have been shown to secrete axon guidance molecules such as netrins (16, 17), and it could be hypothesized that these molecules also contribute to facilitate nerve infiltration. Overall, neurotrophic growth factor receptors and neurotransmitter receptors (NTR), such as β-adrenergic receptors, are expressed in both neurons and cancer cells, and their corresponding ligands act as messengers between the nervous system and cancer. This opens new perspectives to revisit the role of neurotrophic growth factors and axon guidance molecules in cancer and their potential value as new oncology treatment target. An overview of nerve–cancer cell cross-talk is presented in Fig. 1.

Potential Antineurogenic Therapies in Cancer

From a therapeutic perspective, the studies by Magnon and colleagues (6) and Zhao and colleagues (7) have shown that targeting nerve fibers in prostate and gastric cancer can inhibit tumor growth and metastasis and could therefore be of clinical interest. However, the drugs used in these investigations, such as 6-hydroxydopamine (6-OHDA) and botulinum toxin, as well as other neurotoxic drugs, are unlikely to be of clinical use as they cross the blood brain barrier and are highly toxic to the central nervous system. There are also issues about the specificity as neurotoxic drugs can eventually impact on non-neuronal cells and tissues. Therefore, inhibiting nerve infiltration without inducing neuronal and non-neuronal toxicity is of great importance to future translation of this discovery to the clinic, and the recent identification of neurotrophic factors as drivers of cancer innervation (14, 15) offers a rationale for new therapeutic strategies.

To date, most strategies for targeting neurotrophic factors have been developed for NGF. As NGF plays an important role in generation of pain (18), blocking antibodies, small pharmacologic inhibitors, and peptides have been designed to antagonize this growth factor and its receptors TrkA and $p75^{NTR}$ (19). In

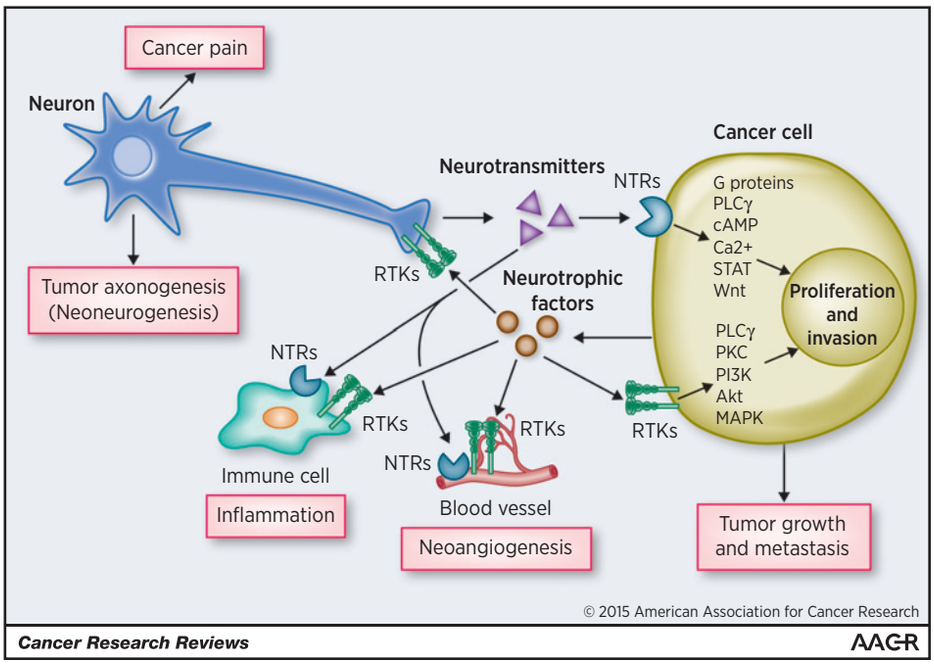

Figure 1.
Nerve–cancer cell cross-talk. Nerves infiltrate the tumor microenvironment and stimulate cancer cell growth and metastasis through the secretion of neurotransmitters (such as catecholamines, acetylcholine, and neuropeptides), initiating signaling pathways for growth and invasion in cancer cells after binding to NTRs. Conversely, nerve infiltration in the tumor is mediated through the liberation of neurotrophic growth factors (such as NGF) by cancer cells, resulting in neuron outgrowth (axonogenesis or neo-neurogenesis), as well as autocrine stimulation of cancer cells via the stimulation of corresponding receptor tyrosine kinases (RTK). This reciprocal interaction fuels tumor development and also impacts the microenvironment, as the liberated neurotransmitters and growth factors can also act on endothelial and immune cells, then contributing to tumor inflammation and neo-angiogenesis. Cancer-induced pain can also be a consequence of tumor innervation. PLCγ, phospholipase C γ; cAMP, cyclic adenosine monophosphate; PKC, protein kinase C.

particular, a humanized monoclonal antibody (tanezumab) is already in clinical trials for its analgesic activity in chronic rheumatoid and back pain. Interestingly, in murine models, anti-NGF antibodies have been shown to decrease pain caused by bone metastases of prostate cancer and to attenuate bone destruction (20, 21). Therefore targeting proNGF/NGF, and more generally neurotrophic factors, could also have an additional positive impact by reducing cancer-associated pain, but this perspective warrants further preclinical and clinical investigations. To date, antineurogenic therapies have been tested for the treatment of pain and neurologic diseases, but they could now be redirected to oncology treatments to inhibit nerve infiltration in cancer. In addition, antineurogenic therapies could have a direct effect on cancer cells, as many cancer cells respond to neurotrophic growth factors, such as NGF, by increasing proliferation and migration via the activation of the corresponding tyrosine kinase receptors. This has been particularly well described for neurotrophins in breast cancer (22). For instance, NGF and proNGF are expressed in breast cancer and stimulate tumor cell growth and invasion (23, 24), including the stem cell compartment (25). In prostate cancer, changes in the expression of NGF and its Trk receptor contribute to tumor cell growth and dissemination through a variety of kinase-based signaling pathways, and the inhibition of Trk receptors decreases the growth of prostatic cancer cells (26). Although neurotrophic factors are less studied in gastric cancer, a recent investigation has reported that the expression of brain-derived neurotrophic growth factor and its receptor TrkB in gastric cancer cells strongly contributes to gastric cancer cell proliferation and dissemination (27). Therefore, targeting neurotrophic growth factors in cancer would have an impact on tumor progression via the inhibition of nerve infiltration, but also through a direct targeting of cancer cells.

Future Directions

Following this major advance, many questions are being generated by the discovery of nerve involvement in prostate and gastric cancers. How generalizable is nerve involvement in cancer progression? The microenvironment of colorectal cancer for instance is rich in autonomic nerve fibers and the presence of nerves has recently been associated with shortened patient survival in colon cancer (28). However, the potential impact of nerves on colorectal cancer progression has not been reported and therefore it is still to be determined whether a similar nerve-dependent tumor growth takes place. In breast cancer, it has been shown that the sympathetic nervous system can induce a metastatic switch (29), but a possible relationship with nerve fiber infiltration in breast tumors has yet to be established. Another potentially important question relates to the impact of β-blockers on survival of patients with cancer. It has been suggested, mainly in breast (30) and in prostate cancer (31), that blockers of the β-adrenergic receptor, traditionally used for the treatment of cardiovascular disorders and anxiety, might increase cancer patient survival. However, the mechanism is not resolved and it

may be hypothesized that beta-blockers actually inhibit the stimulatory effect of catecholamines liberated by nerves in the tumor microenvironment. In addition, the effect of beta-blockers may also be related to the inhibition of stress, as dopamine, a stress inhibitory catecholamine has been shown to decrease ovarian cancer growth though an antiangiogenic effect (32, 33). Finally, to date, only sympathetic and parasympathetic nerves have been implicated in tumor progression and the role of sensory nerves has not yet been reported. Sensory nerves can eventually innervate primary tumors and metastases, thus contributing to tumor-associated pain as demonstrated in pancreatic (5) and prostate cancers (21).Therefore, a possible involvement of sensory fibers in tumor progression, although not demonstrated at this stage, cannot be excluded.

Conclusion

The recent developments described in this review demonstrate that the interaction between nerves and cancer cells goes far beyond the concept of perineural invasion and pain. Nerve infiltration in the tumor microenvironment plays an essential role in both stimulating cancer cell growth and metastasis. These advances bridge a critical gap in knowledge regarding the interplay between the nervous system and cancer, thus opening the way to future investigation of the neurobiology of cancer. The nerve–cancer cell cross-talk opens new therapeutic perspectives in oncology and future development of antineurogenic strategies should be eagerly anticipated.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported by University of Newcastle Australia and Hunter Cancer Research Alliance.

Received October 27, 2014; revised November 30, 2014; accepted December 16, 2014; published OnlineFirst March 20, 2015.

References

1. De Sousa Pereira A. A basis for sympathectomy for cancer of the cervix uteri. Arch Surg 1946;52:260–85.
2. Barnbrook DH. Pheochromocytoma; an alternative surgical approach. Can Med Assoc J 1953;68:245–7.
3. Batkin S, Piette LH, Wildman E. Effect of muscle denervation on growth of transplanted tumor in mice. Proc Natl Acad Sci U S A 1970;67: 1521–7.
4. Marchesi F, Piemonti L, Mantovani A, Allavena P. Molecular mechanisms of perineural invasion, a forgotten pathway of dissemination and metastasis. Cytokine Growth Factor Rev 2010;21:77–82.
5. Bapat AA, Hostetter G, Von Hoff DD, Han H. Perineural invasion and associated pain in pancreatic cancer. Nat Rev Cancer 2011;11:695–707.
6. Magnon C, Hall SJ, Lin J, Xue X, Gerber L, Freedland SJ, et al. Autonomic nerve development contributes to prostate cancer progression. Science 2013;341:1236361.
7. Zhao CM, Hayakawa Y, Kodama Y, Muthupalani S, Westphalen CB, Andersen GT, et al. Denervation suppresses gastric tumorigenesis. Sci Transl Med 2014;6:250ra115.
8. Gross ER, Gershon MD, Margolis KG, Gertsberg ZV, Li Z, Cowles RA. Neuronal serotonin regulates growth of the intestinal mucosa in mice. Gastroenterology 2012;143:408–17 e2.
9. Thaker PH, Han LY, Kamat AA, Arevalo JM, Takahashi R, Lu C, et al. Chronic stress promotes tumor growth and angiogenesis in a mouse model of ovarian carcinoma. Nat Med 2006;12:939–44.
10. Kim-Fuchs C, Le CP, Pimentel MA, Shackleford D, Ferrari D, Angst E, et al. Chronic stress accelerates pancreatic cancer growth and invasion: a critical role for beta-adrenergic signaling in the pancreatic microenvironment. Brain Behav Immun 2014;40:40–7.
11. Melamed R, Rosenne E, Shakhar K, Schwartz Y, Abudarham N, Ben-Eliyahu S. Marginating pulmonary-NK activity and resistance to experimental tumor metastasis: suppression by surgery and the prophylactic use of a beta-adrenergic antagonist and a prostaglandin synthesis inhibitor. Brain Behav Immun 2005;19:114–26.
12. Li ZJ, Cho CH. Neurotransmitters, more than meets the eye–neurotransmitters and their perspectives in cancer development and therapy. Eur J Pharmacol 2011;667:17–22.
13. Wang H, Yu M, Ochani M, Amella CA, Tanovic M, Susarla S, et al. Nicotinic acetylcholine receptor alpha7 subunit is an essential regulator of inflammation. Nature 2003;421:384–8.
14. Pundavela J, Demont Y, Jobling P, Lincz LF, Roselli S, Thorne RF, et al. ProNGF correlates with Gleason score and is a potential driver of nerve infiltration in prostate cancer. Am J Pathol 2014;184:3156–62.
15. Dobrenis K, Gauthier LR, Barroca V, Magnon C. Granulocyte colony-stimulating factor off-target effect on nerve outgrowth promotes prostate cancer development. Int J Cancer 2015; 136:982–8.
16. Mehlen P, Delloye-Bourgeois C, Chedotal A. Novel roles for Slits and netrins: axon guidance cues as anticancer targets? Nat Rev Cancer 2011;11:188–97.
17. Biankin AV, Waddell N, Kassahn KS, Gingras MC, Muthuswamy LB, Johns AL, et al. Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature 2012;491:399–405.
18. Pezet S, McMahon SB. Neurotrophins: mediators and modulators of pain. Annu Rev Neurosci 2006;29:507–38.
19. Longo FM, Massa SM. Small-molecule modulation of neurotrophin receptors: a strategy for the treatment of neurological disease. Nat Rev Drug Discov 2013;12:507–25.
20. Jimenez-Andrade JM, Ghilardi JR, Castaneda-Corral G, Kuskowski MA, Mantyh PW. Preventive or late administration of anti-NGF therapy attenuates tumor-induced nerve sprouting, neuroma formation, and cancer pain. Pain 2011;152:2564–74.
21. McCaffrey G, Thompson ML, Majuta L, Fealk MN, Chartier S, Longo G, et al. NGF blockade at early times during bone cancer development attenuates bone destruction and increases limb use. Cancer Research 2014;74:7014–23.
22. Hondermarck H. Neurotrophins and their receptors in breast cancer. Cytokine Growth Factor Rev 2012;23:357–65.
23. Adriaenssens E, Vanhecke E, Saule P, Mougel A, Page A, Romon R, et al. Nerve growth factor is a potential therapeutic target in breast cancer. Cancer Res 2008;68:346–51.
24. Demont Y, Corbet C, Page A, Ataman-Onal Y, Choquet-Kastylevsky G, Fliniaux I, et al. Pro-nerve growth factor induces autocrine stimulation of breast cancer cell invasion through tropomyosin-related kinase A (TrkA) and sortilin protein. J. Biol Chem 2012;287:1923–31.
25. Tomellini E, Touil Y, Lagadec C, Julien S, Ostyn P, Zietal-Gelus N, et al. NGF and proNGF simultaneously promote symmetric self-renewal, quiescence and EMT to enlarge the breast cancer stem cell compartment. Stem Cells 2015;33:342–53.
26. Miknyoczki SJ, Wan W, Chang H, Dobrzanski P, Ruggeri BA, Dionne CA, et al. The neurotrophin-trk receptor axes are critical for the growth and progression of human prostatic carcinoma and pancreatic ductal adenocarcinoma xenografts in nude mice. Clin Cancer Res 2002;8: 1924–31.
27. Okugawa Y, Tanaka K, Inoue Y, Kawamura M, Kawamoto A, Hiro J, et al. Brain-derived neurotrophic factor/tropomyosin-related kinase B pathway in gastric cancer. Br J Cancer 2013;108:121–30.
28. Liebl F, Demir IE, Rosenberg R, Boldis A, Yildiz E, Kujundzic K, et al. The severity of neural invasion is associated with shortened survival in colon cancer. Clinical Cancer Research 2013;19:50–61.
29. Sloan EK, Priceman SJ, Cox BF, Yu S, Pimentel MA, Tangkananggnukul V, et al. The sympathetic nervous system induces a

metastatic switch in primary breast cancer. Cancer Res 2010;70: 7042–52.
30. Barron TI, Connolly RM, Sharp L, Bennett K, Visvanathan K. Beta blockers and breast cancer mortality: a population- based study. J. Clin Oncol 2011;29:2635–44.
31. Grytli HH, Fagerland MW, Fossa SD, Tasken KA. Association between use of beta-blockers and prostate cancer-specific survival: a cohort study of 3561 prostate cancer patients with high-risk or metastatic disease. European Urology 2014;65:635–41.
32. Moreno-Smith M, Lu C, Shahzad MM, Pena GN, Allen JK, Stone RL, et al. Dopamine blocks stress-mediated ovarian carcinoma growth. Clin Cancer Res 2011;17:3649–59.
33. Moreno-Smith M, Lee SJ, Lu C, Nagaraja AS, He G, Rupaimoole R, et al. Biologic effects of dopamine on tumor vasculature in ovarian carcinoma. Neoplasia 2013;15:502–10.
34. Li X, Ma G, Ma Q, Li W, Liu J, Han L, et al. Neurotransmitter substance P mediates pancreatic cancer perineural invasion via NK-1R in cancer cells. Mol Cancer Res 2013;11:294–302.