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Commentary

Clinically Actional Strategies for Studying Neural Influences in Cancer

Ihsan Ekin Demir,[1,2,3,4,*] Carmen Mota Reyes,[1,3,4] Wasfi Alrawashdeh,[5] Güralp O. Ceyhan,[2] Sylvie Deborde,[6] Helmut Friess,[1,3,4] Kivanc Görgülü,[7] Rouzanna Istvanffy,[1,3,4] David Jungwirth,[1,3,4] Rohini Kuner,[8] Maria Maryanovich,[9,10] Shorook Na'ara,[11,12] Simon Renders,[13,14,15] Jami L. Saloman,[16] Nicole N. Scheff,[17] Hendrik Steenfadt,[1,3,4] Pavel Stupakov,[1,3,4] Vera Thiel,[13,14,15] Divij Verma,[9,10] Bengi Su Yilmaz,[1,3,4] Ruth A. White,[18] Timothy C. Wang,[19] Richard J. Wong,[6] Paul S. Frenette,[9,10,20] Ziv Gil,[11,12] and Brian M. Davis[21]

[1]Department of Surgery, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany
[2]Department of General Surgery, HPB-Unit, School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[3]German Cancer Consortium (DKTK), Partner Site Munich, Munich, Germany
[4]CRC 1321 Modelling and Targeting Pancreatic Cancer, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany
[5]Department of HPB and Transplant Surgery, The Freeman Hospital, Newcastle upon Tyne, Tyne and Wear, UK
[6]Department of Surgery, Memorial Sloan Kettering Cancer Center, New York, NY, USA
[7]Comprehensive Cancer Center Munich, Klinikum rechts der Isar, Technical University of Munich, School of Medicine, Munich, Germany
[8]Department of Molecular Pharmacology, Institute of Pharmacology, Heidelberg University, Heidelberg, Germany
[9]Ruth L. and David S. Gottesman Institute for Stem Cell and Regenerative Medicine Research, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[10]Department of Cell Biology, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[11]Department of Otolaryngology, Head and Neck Surgery, and the Laboratory for Applied Cancer Research, Rappaport Institute of Medicine and Research, The Technion, Israel Institute of Technology, Haifa, Israel
[12]Head and Neck Center, Rambam Healthcare Campus, Haifa, Israel
[13]Division of Stem Cells and Cancer, German Cancer Research Center (DKFZ) and DKFZ-ZMBH Alliance, 69120 Heidelberg, Germany
[14]Heidelberg Institute for Stem Cell Technology and Experimental Medicine (HI-STEM gGmbH), 69120 Heidelberg, Germany
[15]Department of Internal Medicine V, Hematology, Oncology and Rheumatology, University Hospital Heidelberg, Heidelberg, Germany
[16]Department of Medicine, Division of Gastroenterology, Hepatology, & Nutrition, Center for Neuroscience at the University of Pittsburgh, Pittsburgh Center for Pain Research and Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[17]Hillman Cancer Center and Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
[18]Division of Hematology and Oncology, Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA
[19]Division of Digestive and Liver Diseases, Department of Medicine, Columbia University, College of Physicians and Surgeons, New York, NY, USA
[20]Department of Medicine, Albert Einstein College of Medicine, Bronx, NY 10461, USA
[21]Center for Neuroscience at the University of Pittsburgh, Pittsburgh Center for Pain Research and Department of Neurobiology, University of Pittsburgh School of Medicine, Pittsburgh, PA, USA
*Correspondence: ekin.demir@tum.de
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Neuro-glial activation is a recently identified hallmark of growing cancers. Targeting tumor hyperinnervation in preclinical and small clinical trials has yielded promising antitumor effects, highlighting the need of systematic analysis of neural influences in cancer (NIC). Here, we outline the strategies translating these findings from bench to the clinic.

Cancer cells induce sprouting of peripheral nerve fibers termed "axonogenesis" (Boilly et al., 2017); its underlying mechanism is thought to be similar to cancer's ability to initiate angiogenesis. Increased and functionally altered innervation has been shown to be associated with worse prognosis in several cancers, such as pancreatic (Ceyhan et al., 2009), prostate (Magnon et al., 2013), gastric (Zhao et al., 2014), colorectal (Albo et al., 2011), head and neck (Gil et al., 2007), and hematological cancers (Hanoun et al., 2014). Exploitation of neuronal activity by cancer cells has recently been viewed as a central and common pathomechanism for progression in both solid and hematological cancers and has emerged as a dynamic research field at the intersection of oncology and neuroscience.

Given the recent advances made in the study of neural influences in cancer (NIC), it is now the time to define concrete, clinically actionable strategies for targeting NIC and for turning axonogenesis into an oncologically relevant target, similar to angiogenesis (Figure 1).

Targeting NIC: What Is the Therapeutic Potential?

Denervation as a Therapeutic Approach in Cancer

There is accumulating evidence for the dependence of growing cancers on innervation. Numerous animal studies have convincingly shown that complete or selective surgical, pharmacological, or genetic denervation of solid cancers (Magnon et al., 2013; Saloman et al., 2016; Zhao et al., 2014) leads to deceleration of tumor growth.

Denervation approaches have taught us the pathophysiology of NIC, yet translating denervation approaches into the human setting might be technically difficult. There are examples of successful attenuation of cancer progression following chemical denervation during surgery (Lillemoe et al., 1993) but also failed trials without effect on cancer growth despite promising preclinical findings (ClinicalTrials.gov Identifier: NCT01520441). Denervation may be

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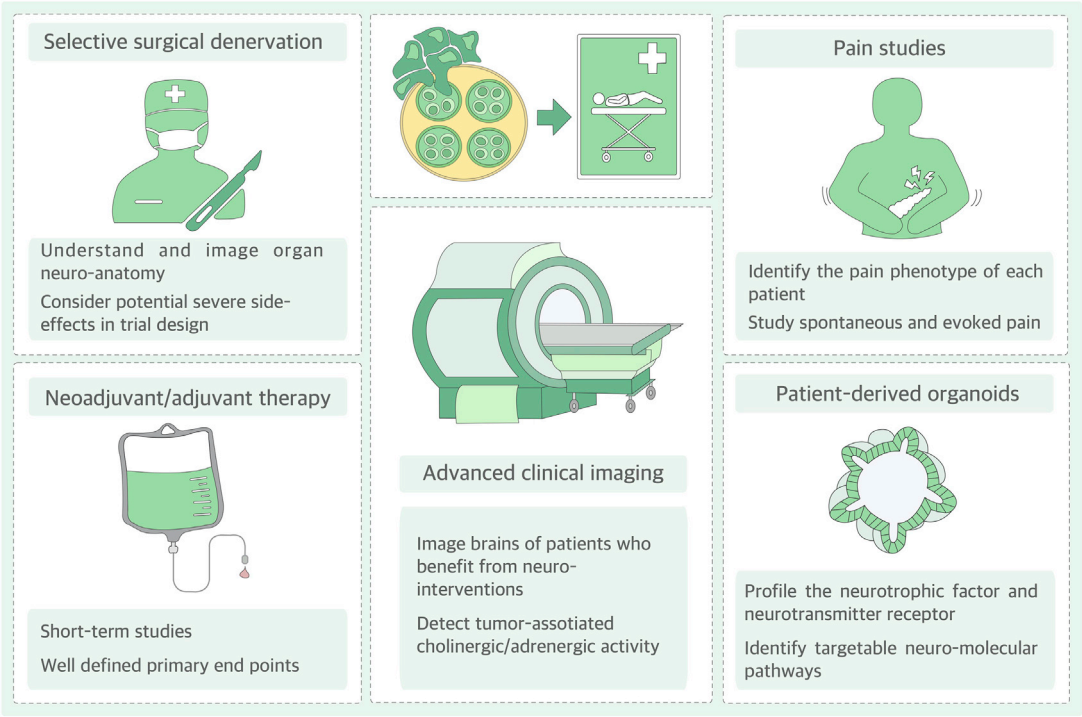

Figure 1. Bench-to-Clinic Strategies Related to Neural Influences in Cancer (NIC)
Here we propose five strategies to translate the recent findings from the NIC field into a clinically applicable stage.

effective over a limited time period in tumor pathogenesis, given the complex, multifactorial nature of the tumor milieu. Furthermore, there are key anatomic considerations for each organ. For organs like the colon (especially its more carcinotropic part, i.e., the left hemicolon) or rectum, which receive a complex innervation from multiple extrinsic (vagus nerve, hypogastric plexus, and sacral plexus) and intrinsic (enteric nervous system [ENS]) sources, it may be difficult to identify the primary driver of nerve-cancer interactions in an organ-specific manner (Graham et al., 2020). The side effects of denervation, as known from radical resection approaches for cancers directly infiltrating neural plexus (e.g., pancreatic or prostate cancer), can be associated with severe postoperative complaints such as intractable diarrhea, sexual dysfunction, or incontinence. Similarly, denervation in tumors of the head and neck region (e.g., tongue and/or oropharyngeal cancers) can be associated with severe organ dysfunction. Therefore, future clinical studies that plan to perform such selective denervation approaches in cancer patients should consider the potential ethical and clinical consequences of these innovative techniques on patient quality of life.

Targeting Autonomic Nervous Activity

An alternative approach for modulating neural activity in cancers may be to repurpose medications known to regulate sympathetic or parasympathetic signaling, such as selective or non-selective beta blockers (propranolol or metoprolol), or parasympathomimetic agents (bethanechol) as adjuvant therapy for cancer patients. Here, it is important to consider the differences in the cholinergic response among cancers, such as gastric versus pancreatic cancer. Indeed, a number of retrospective studies have shown improved outcomes for patients taking beta blockers in a number of cancer types (Qiao et al., 2018). New clinical trials are emerging targeting the autonomic nervous system with muscarinic agonists (NCT03572283) and beta blockers in both non-metastatic patients (perioperative use) or metastatic patients (advanced disease) (NCT02944201, NCT03838029, and NCT04245644). Despite these advances, large randomized controlled trials are currently lacking and will be needed to demonstrate a benefit from these agents.

Molecular Targets Pertinent to NIC

It is imperative to identify the specific target molecules relevant for the impact of the nervous system in different cancers. In addition to generating more specific drug inhibitors or activators (e.g., adrenergic and cholinergic receptors), it seems highly relevant to generate and apply specific molecular inhibitors of the protumorigenic neural pathways. Examples for such neuron- or glia-specific classes of molecules are neurotrophic factors and neurotransmitters with their corresponding receptors. For example, targeting the nerve growth factor (NGF) signaling via tanezumab is an effective method for analgesia and bears the potential of treating NGF-overexpressing cancers (Demir et al., 2016). Tyrosine kinase receptors expressed by cancer cells are also potential targets to prevent neural dissemination by cancer (Amit et al., 2016). There is an urgent need to identify specific neuron- or glia-derived mediators of nerve-cancer interactions across cancer types. In-depth profiling of the transcriptomic, proteomic, and epigenetic

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signature of tumors at the single-cell level will help achieve this goal.

Strategies for Future Bench-to-Clinic Studies on NIC

Integrate Oncologists

Although NIC is a relative young field of research, the available findings already allow the performance of clinical trials. Indeed, such trials that make use of beta-blockers, botulinum toxin, or betanechol, for example, are already running, and the first results are expected within the next three years. We recommend the consideration of a simple, rather than complex, clinical study design. To see the effectiveness of co-administration of neuro-modulatory drugs with other established anti-cancer agents, clear-cut primary endpoints are necessary. For solid tumors, one can consider the administration of the agents in neoadjuvant (preoperative) setting, and the primary endpoint can be the margin-free (R0) resection rate, the extent of perineural invasion (Pn), or lymph node metastasis (N status). Such short-term study designs can not only have major impact on the prognosis of the patients but also lay the foundation for subsequent large-scale, long-term oncological studies.

Integrate Surgeons

For solid tumors, surgeons have the unique opportunity to directly expose the tumor during surgical exploration. This approach enables several maneuvers such as selective surgical denervation (anterior or posterior vagotomy or sympathectomy), chemical denervation (botulinum toxin injection into the tumor), or the local direct injection of neuro-modulatory agents or placement of neuro-stimulatory devices around or into the tumor. For solid tumors, such surgical maneuvers should be considered for downsizing the tumors that are initially found to be unresectable.

Profile Nervous-System-Related Targets on Cancer Cells

Organoids grown in a 3D culture environment can better mimic the natural activation status and molecular signature of cancer cells. Molecular profiling of patient-derived organoids (PDOs) is currently being tested within clinical trials for identifying patient-specific molecular vulnerabilities of cancer cells. For some cancers, genetic alterations in nervous-system-related pathways (e.g., the axon guidance genes *Slit*/*Robo*) are among the most prominently activated molecular signatures (Biankin et al., 2012). Identifying the individual "neuro-molecular profile" of patients may yield valuable clues for individualized, neuro-targeting therapies for specific patient groups.

Advance Clinical Imaging Technologies

Advanced imaging technologies such as positron-emission tomography (PET) using specific neuro-molecular targets can be very useful to identify patients who exhibit, for example, cholinergic activity in the tumor or at distant sites. The uptake of PET tracers binding to cholinergic nerves (i.e., (11)C-donepezil and (18)F-FEOBV) is considerably higher in prostate cancer tissues with a high severity (Gleason) score (Stokholm et al., 2016). Development of novel neuro-molecular tracers and performance of such specific neuro-tracer-PET imaging within clinical studies may help identify patients who may specifically benefit from neuro-modulatory therapies.

Study Cancer-Associated Pain

Studying pain in the context of human cancer requires detailed prospective analyses comprising not only questionnaires but also quantitative sensory testing as well as diligent attention to the choice of the correct preclinical models for reverse translation. While pain studies in human patients reveal pain phenotypes and their temporal relation to dynamic tumor progression, animal models allow the identification of underlying mechanisms. With the large number of available transgenic mouse lines, it is possible to study individual genes in cancer-associated pain behavior. However, behavioral analyses in animal models require significant experience to ensure data reproducibility. Both evoked hypersensitivity and spontaneous pain behaviors should be assessed, as the potential pharmacology to treat evoked versus breakthrough pain can differ. The contribution of sensory neuron subpopulations and corresponding sensory neurotransmission in animal models may differ in patients; therefore, target proteins and neuron subpopulations should be verified in human specimens. In addition, in certain cancers, there is a strong variation of pain-associated behavior depending on the cancer stage. The pain signaling in the early stages may be masked by the opioid signaling mediated by the immune system and the endogenous anti-nociceptive pathways (e.g., desceding modulation). These pathways may be overwhelmed later in the disease, leading to aggressive, difficult-to-treat breakthrough pain that may be driven by a combination of inflammatory and neuropathic pain features. Assessing differences in painful versus painless cancers may reveal important clues to pain control in cancer.

Conclusions

Dependence of cancer cells on nerves for their evolution and spread and the promising anti-cancer effects of surgical or pharmacological denervation or modulation approaches have provided a solid basis for future translational investigations in the NIC field. Here, we outline key clinically actionable strategies for advancing the NIC field into a "clinically applicable" stage. Upon successful implementation of these strategies, axonogenesis research may contribute to strong clinical utility similar to angiogenesis research. The NIC group is actively calling for integration of talented scientists and clinicians worldwide into this collaborative effort.

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REFERENCES

Albo, D., Akay, C.L., Marshall, C.L., Wilks, J.A., Verstovsek, G., Liu, H., Agarwal, N., Berger, D.H., and Ayala, G.E. (2011). Neurogenesis in colorectal

cancer is a marker of aggressive tumor behavior and poor outcomes. Cancer *117*, 4834–4845.

Amit, M., Na'ara, S., and Gil, Z. (2016). Mechanisms of cancer dissemination along nerves. Nat. Rev. Cancer *16*, 399–408.

Biankin, A.V., Waddell, N., Kassahn, K.S., Gingras, M.C., Muthuswamy, L.B., Johns, A.L., Miller, D.K., Wilson, P.J., Patch, A.M., Wu, J., et al.; Australian Pancreatic Cancer Genome Initiative (2012). Pancreatic cancer genomes reveal aberrations in axon guidance pathway genes. Nature *491*, 399–405.

Boilly, B., Faulkner, S., Jobling, P., and Hondermarck, H. (2017). Nerve Dependence: From Regeneration to Cancer. Cancer Cell *31*, 342–354.

Ceyhan, G.O., Bergmann, F., Kadihasanoglu, M., Altintas, B., Demir, I.E., Hinz, U., Muller, M.W., Giese, T., Buchler, M.W., Giese, N.A., and Friess, H. (2009). Pancreatic neuropathy and neuropathic pain–a comprehensive pathomorphological study of 546 cases. Gastroenterology *136*, 177–186.

Demir, I.E., Tieftrunk, E., Schorn, S., Friess, H., and Ceyhan, G.O. (2016). Nerve growth factor & TrkA as novel therapeutic targets in cancer. Biochim. Biophys. Acta *1866*, 37–50.

Gil, Z., Rein, A., Brader, P., Li, S., Shah, J.P., Fong, Y., and Wong, R.J. (2007). Nerve-sparing therapy with oncolytic herpes virus for cancers with neural invasion. Clin. Cancer Res. *13*, 6479–6485.

Graham, K.D., Lopez, S.H., Sengupta, R., Shenoy, A., Schneider, S., Wright, C.M., Feldman, M., Furth, E., Valdivieso, F., Lemke, A., et al. (2020). Robust, 3-Dimensional Visualization of Human Colon Enteric Nervous System Without Tissue Sectioning. Gastroenterology. Published online February 27, 2020. https://doi.org/10.1053/j.gastro.2020.02.035.

Hanoun, M., Zhang, D., Mizoguchi, T., Pinho, S., Pierce, H., Kunisaki, Y., Lacombe, J., Armstrong, S.A., Dührsen, U., and Frenette, P.S. (2014). Acute myelogenous leukemia-induced sympathetic neuropathy promotes malignancy in an altered hematopoietic stem cell niche. Cell Stem Cell *15*, 365–375.

Lillemoe, K.D., Cameron, J.L., Kaufman, H.S., Yeo, C.J., Pitt, H.A., and Sauter, P.K. (1993). Chemical splanchnicectomy in patients with unresectable pancreatic cancer. A prospective randomized trial. Ann. Surg. *217*, 447–455, discussion 456–457.

Magnon, C., Hall, S.J., Lin, J., Xue, X., Gerber, L., Freedland, S.J., and Frenette, P.S. (2013). Autonomic nerve development contributes to prostate cancer progression. Science *341*, 1236361.

Qiao, G., Chen, M., Bucsek, M.J., Repasky, E.A., and Hylander, B.L. (2018). Adrenergic Signaling: A Targetable Checkpoint Limiting Development of the Antitumor Immune Response. Front. Immunol. *9*, 164.

Saloman, J.L., Albers, K.M., Li, D., Hartman, D.J., Crawford, H.C., Muha, E.A., Rhim, A.D., and Davis, B.M. (2016). Ablation of sensory neurons in a genetic model of pancreatic ductal adenocarcinoma slows initiation and progression of cancer. Proc. Natl. Acad. Sci. USA *113*, 3078–3083.

Stokholm, M.G., Høyer, S., Borre, M., Bender, D., Jakobsen, S., Frøkiær, J., and Borghammer, P. (2016). Molecular imaging of cholinergic processes in prostate cancer using ^{11}C-donepezil and ^{18}F-FEOBV. Eur. J. Nucl. Med. Mol. Imaging *43*, 906–910.

Zhao, C.M., Hayakawa, Y., Kodama, Y., Muthupalani, S., Westphalen, C.B., Andersen, G.T., Flatberg, A., Johannessen, H., Friedman, R.A., Renz, B.W., et al. (2014). Denervation suppresses gastric tumorigenesis. Sci. Transl. Med. *6*, 250ra115.