Oral Oncology 47 (2011) 1005–1010

Contents lists available at SciVerse ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

Review

Perineural invasion in oral squamous cell carcinoma: A discussion of significance and review of the literature

Nada O. Binmadi [a,b], John R. Basile [a,c,*]

[a] Department of Oncology and Diagnostic Sciences, University of Maryland Dental School, 650 West Baltimore Street, 7-North, Baltimore, MD 21201, USA
[b] Department of Oral Basic & Clinical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia
[c] Marlene and Stuart Greenebaum Cancer Center, 22 South Greene Street, Baltimore, MD 21201, USA

ARTICLE INFO

Article history:
Received 20 May 2011
Received in revised form 27 July 2011
Accepted 1 August 2011
Available online 23 August 2011

Keywords:
Oral squamous cell carcinoma
Perineural invasion
Metastasis
Neurotropic carcinoma

SUMMARY

Perineural invasion (PNI) is a tropism of tumor cells for nerve bundles in the surrounding stroma. It is a form of tumor spread exhibited by neurotropic malignancies that correlates with aggressive behavior, disease recurrence and increased morbidity and mortality. Oral squamous cell carcinoma (OSCC) is a neurotropic malignancy that traditionally has been difficult to treat and manage. Evidence suggests that demonstration of PNI in OSCC should impact adjuvant treatment decisions and surgical management of this disease. Despite its importance as a prognostic indicator, experimental studies to explore the molecular mechanisms responsible for PNI are limited. The aim of this review is to discuss the difficulties in evaluating for PNI, review the literature regarding the relationship of PNI with patient outcomes in OSCC, and summarize the recent studies describing the molecular agents associated with this pathological phenomenon.

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Introduction

Oral squamous cell carcinoma (OSCC) is the sixth most common malignancy worldwide and encompasses at least 90% of all oral malignancies. OSCC is associated with severe disease and treatment-related morbidity and is often reported as having high rates of recurrence and poor disease-free survival despite advances in cancer treatment.[1] However, recent studies do show some improvement in outcomes following primary surgery, depending upon the site of the lesion and the use of more aggressive therapy such as elective neck dissection.[2,3] When difficulties managing these patients do arise, it is often because of occurrence of regional or distant metastatic spread of their disease.[4] Like other epithelial malignancies, OSCC is a heterogeneous group of tumors that arises from the accumulation of a series of genetic and epigenetic alterations, usually from exposure to tobacco-associated carcinogens, resulting in the activation of oncogenes and inactivation of tumor suppressors. These genetic changes confer proliferation and survival advantages to the altered cells, characterized by growth factor-independent cell division, resistance to apoptotic signaling and an enhanced capacity to degrade and move through the tissues of the extracellular matrix and invade adjacent structures. The ability of cells of a carcinoma to break through the basal lamina, liberate themselves from the primary lesion, avoid host defenses, gain access to lymphatics or the circulation, and establish a new growing lesion at a distant site is the basis for metastasis and represents one of the most difficult barriers to overcome in the treatment of oral cancer.[5]

Another quality possessed by certain tumors, referred to as 'neurotropic malignancies,' is perineural invasion (PNI). PNI is a tropism of tumor cells for nerve bundles in the surrounding tissues. PNI is a form of metastatic tumor spread similar to but distinct from vascular or lymphatic invasion that hinders the ability to establish local control of a malignancy because neoplastic cells can travel along nerve tracts far from the primary lesion and are often missed during surgery.[6] As a result, these tumors can exhibit pain and persistent growth with a long clinical course and late onset of metastases, a pattern that has been observed in neurotropic tumor types such as melanoma, prostate and pancreatic cancer and the salivary gland malignancies adenoid cystic carcinoma and polymorphous low-grade adenocarcinoma.[7–9] Among the various parameters used to predict the outcome of malignant disease, PNI is in wide use as an indicator of aggressive behavior.[5] PNI is well known as an independent predictor of poor outcome in colorectal carcinoma and salivary gland malignancies.[9–11] The purpose of this review is to draw attention to OSCC as a neurotropic malignancy and review the findings in the literature that describe this phenomenon as it relates to mechanism, treatment and disease prognosis.

* Corresponding author at: Department of Oncology and Diagnostic Sciences, University of Maryland Dental School, 650 West Baltimore Street, 7-North, Baltimore, MD 21201, USA. Tel.: +1 410 706 7936; fax: +1 410 706 0519.
E-mail address: jbasile@umaryland.edu (J.R. Basile).

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doi:10.1016/j.oraloncology.2011.08.002

Mechanism and histopathological assessment of PNI

Cruveilheir was the first to recognize PNI in head and neck cancer in 1835.[12] Despite the fact that it has been identified for more than 150 years, the mechanism of PNI is still poorly understood and, to date, no treatments have been developed to target this pathologic entity. Different theories have been proposed to explain the exact nature of PNI. Previously, it was considered to be a mechanical extension of cancer cells along planes of least resistance, for example by proliferation through the loose connective tissue sheath of the perineurium or via the lymphatics of the epineurium.[13] These theories were discarded with the emergence of ultra-structural scans of the nerve sheath which revealed that the perineurium is actually a relatively tight and highly selective barrier separating nerves from surrounding tissue.[14] Tumor cells do not passively grow along nerves but instead penetrate the perineurium in a direct and continuous manner, becoming intimately associated with Schwann cells and axons in the endoneurium.[15] Further studies also have shown that the perineurium and endoneurium are devoid of lymphatic channels.[16] Because it is known that some specific tumor types exhibit characteristic neural invasion, whereas other more aggressive tumors fail to do so even at advanced stages, it is instead likely that there are complex biological interactions between certain cancer cells and nerves that need to be considered when discussing PNI.[17] The perineural space provides a suitable microenvironment for the growth of cells from neurotropic malignancies, probably due to cellular factors and their respective receptors that attract the cancer cells and stimulate their growth along the nerves. cDNA microarrays used to profile differential gene expression in adenoid cystic carcinomas with and without PNI have identified dysregulation of genes associated with cell cycle, the cytoskeleton and cell–extracellular matrix interaction that influence the production of neurotropic factors and adhesion molecules contributing to PNI.[18] It is clear that a better understanding of the molecular and biological mechanisms involved will be necessary if we are to target PNI as part of advanced therapies for cancer.

Neural extension of OSCC can be demonstrated not only by MRI and CT, but also through a thorough histologic examination of biopsied tissue. While both the Royal College of Pathologists in the United Kingdom (http://www.rcpath.org; see: Head and Neck Datasets, Section A) and the College of American Pathologists[19] require recording of the presence or absence of invasion of the perineural space by head and neck carcinomas, particularly when occurring ahead of the invasive front of the tumor, the accuracy of PNI analysis is controversial and open to subjectivity. There is a marked variation in the frequency of PNI reporting, ranging in OSCC from a low of anywhere between 2% and 30%[20–23] to a high of 82%,[23] with

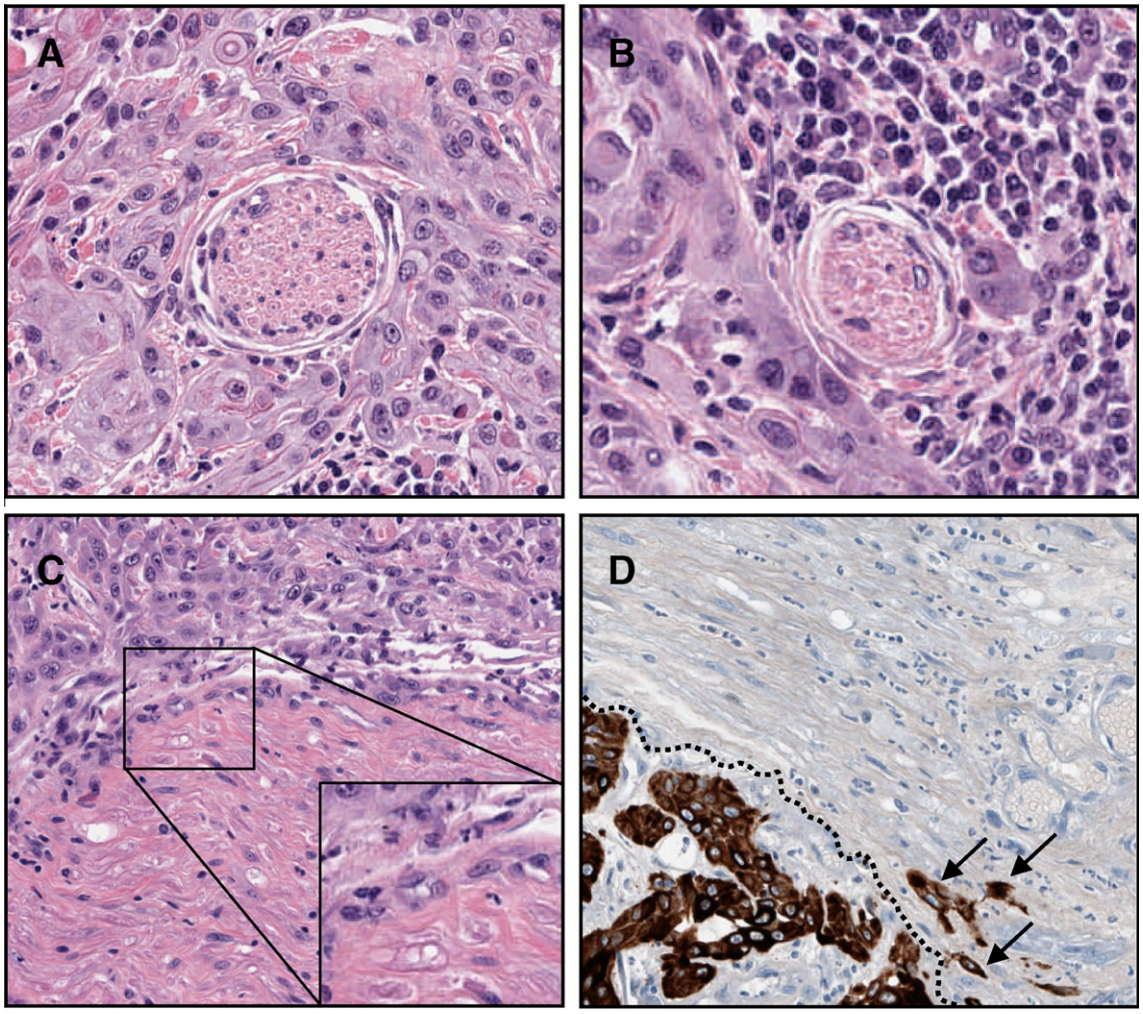

Figure 1 Perineural invasion in OSCC. (A) PNI as defined by Dunn et al.[24] demonstrating malignant cells exhibiting total circumferential involvement of a nerve in a tangential histological section (hematoxylin and eosin stain, original magnification 20×). (B) Based upon the definition of PNI by Liebig et al.[12], tumor cells involving approximately one-third of nerve circumference (hematoxylin and eosin stain, original magnification 20×). (C) The presence of tumor cells within the nerve sheath (hematoxylin and eosin stain; original magnification 20×). (D) Tumor cells inside the nerve sheath, an example of intraneural invasion, as highlighted by pan-cytokeratin stain (black arrows; dotted line represents boundary of the nerve, original magnification 20×).

increasing rates of detection where biopsies are specifically reviewed to detect PNI or when certain neural stains are used. Other concerns in detection of neural invasion in tissue examination are biopsy technique, slide preparation, and the number of histological sections examined. Taken together, these findings suggest that the accuracy and prognostic significance of PNI in many studies of OSCC could be compromised due to errors of underreporting.

A great difficulty exists in that there is no one accepted or standardized definition of PNI among pathologists. Dunn et al. define PNI as the presence of malignant cells in the perineural space with total or near-total circumferential involvement of the nerve in tangential histopathological sections.[24] Liebig et al. have proposed the most widely accepted and referenced description of PNI: (1) tumors in close proximity to a nerve that involve one-third of its circumference and/or (2) the presence of tumor cells within any of the three layers of the nerve sheath (Fig. 1).[12] However, these definitions fail to make a clear distinction between 'perineural' spread, or the discovery of tumor cells in and around the perineural space without infiltration of the nerve fascicle, and 'intraneural' spread, or the penetration of tumor cells within the nerve itself, details that might be difficult to determine histologically but could affect tumor prognosis. Indeed, a study on anterior, middle, and lateral skull base adenoid cystic carcinomas demonstrating PNI found that 39% of the tumors actually showed intraneural invasion.[25] In a study of squamous cell carcinoma of the skin, Mendenhall et al. used the umbrella term 'PNI' for all forms of nerve invasion by tumors, even intraneural spread, due to the fact that intraneural infiltration in their samples was so rare, but failed to study this phenomenon in detail with regards to its significance.[26]

In addition, some clinicians and pathologists are more alarmed by the discovery of PNI away from the tumor invasion front, as opposed to the presence of an intact nerve within the body of a growing tumor. For example, in a study of pancreatic cancers, neural invasion in endocrine tumors was detected only within the boundaries of the tumor itself and not beyond the invasion front, unlike what was observed in pancreatic ductal adenocarcinoma, which the authors believed contributed to the lower rate of local relapse following tumor resection in the endocrine tumors.[27] To our knowledge there are no studies in the literature focusing on details such as the type or quality of PNI in OSCC, particularly as it relates to prognosis, and very little data in other tumor types in general. However, Gil et al. examined this issue in a variety of tumors of the paranasal sinuses, which included some squamous cell carcinomas and salivary gland malignancies, and concluded that neither the pattern of invasion nor the presence of tumor cells directly within nerve bundles had any prognostic significance, though the sample sizes were small.[17]

Relationship between PNI and recurrence, lymph node involvement, tumor stage and age of the patient in OSCC

Prognosis and therefore treatment decisions in OSCC are currently based on TNM staging, as determined by clinical examination, imaging studies, and histopathological features observed in the biopsy that are believed to be risk factors affecting patient outcomes.[28] These factors, which include the pattern of invasion of the tumor, the presence of PNI, and the quality of the lymphocytic response, were shown to be statistically significant independent predictors of both local recurrence and overall survival, regardless of the status of the tumor margins.[28] At least one study failed to find significant differences in 5-year local control and overall survival rates between OSCC patients exhibiting PNI compared to those without,[29] but most investigations have shown that PNI is, to different degrees, associated with disease recurrence, an increased probability of regional and distant metastasis and an overall decrease in 5-year survival rate.[20,22,30–33] Variations in the prognostic importance of a histological finding of PNI among these studies might be due to the size of the nerves involved, with invasion of small nerve branches having a lower correlation with patient outcomes compared to more major nerves. While a review by Woolgar cites evidence that OSCC exhibiting PNI in either major nerves or those of a smaller diameter ($\leqslant$1 mm) are all associated with reduced survival rates and an increased risk of loco-regional recurrence,[31] it is very likely that prognosis worsens when major nerves are involved.[8,11] In adenoid cystic carcinoma, tumors exhibiting PNI in nerves up to 3.0 mm in diameter correlated strongly with tumor size and advanced clinical stage.[34] Prognosis worsens still with the involvement of 'named' nerves.[11] Indeed, where clinical symptoms of encroachment upon the facial nerve are present, such as paresthesia or paralysis, percentages of patients exhibiting metastasis and dying of their disease was greatly increased for both salivary gland malignancies and carcinomas of the skin of the head and neck.[26,35] Due to its anatomical location, OSCC exhibiting PNI into major named nerves also present a unique challenge for the surgeon. Some OSCC have been shown to extend for several centimeters out from the primary lesion.[36] This is particularly a problem in lip squamous cell carcinoma, which has a higher rate of recurrence when exhibiting PNI, is more difficult to control, and can demonstrate intracranial spread through the alveolar, facial and trigeminal nerves and subsequent invasion of the central nervous system, thereby severely limiting treatment options.[26,37,38]

A study in 2007 by Wallwork and co-workers could detect no statistically significant association between PNI (or tumor differentiation) and the presence of lymph node metastases in OSCC of the floor of the mouth.[39] However, the preponderance of evidence in the literature suggests that PNI is a significant prognostic indicator in the ability of OSCC to spread to cervical lymph nodes and therefore should be heavily weighed when considering neck dissection or the use of adjunctive treatment.[30–32,40,41] Larsen et al. demonstrated that nodal involvement at the time of diagnosis of OSCC was significantly related to PNI (as well as grade, the presence of vascular invasion and increasing tumor depth).[42] Tumor grade and PNI were shown to be independent predictors of recurrence and nodal involvement in an Indian study of patients with T1-2, N0 OSCC of the oral anterior tongue.[43] Ross et al. have demonstrated that tumor thickness, a noncohesive invasion front, and neural and bone invasion were all strong histological predictors for cervical lymph node metastases and should be used to avoid underestimation of the presence of occult nodal metastases that can occur in routine clinical and pathological staging.[44]

PNI is correlated with late stage disease. There is a strong tendency toward neural invasion in late stage carcinoma but no association with early stage SCC of the tongue.[45,46] Soudry et al. demonstrated that younger patients suffering from OSCC had a significantly worse N stage, more PNI, and higher rates of treatment failure and mortality when compared to an older patient population.[47] Even though younger patients often have a tendency towards more aggressive disease, and PNI can be a marker of this aggressiveness, Brandwein-Gensler and co-workers could not establish a link between PNI and the age of the patient.[28] There has been some discussion of the link of human papillomavirus (HPV) with the development of OSCC in younger patients, but a recent study has shown that there is no direct impact of HPV or the status of the tumor suppressor p16 on the development of PNI.[48]

Nerve and cancer cell interactions

In the last few years many hypotheses have emphasized the importance of microenvironment for providing the biological and physical parameters necessary to promote PNI. Cancer cell

migration towards nerves and then along the nerve trunk within the perineural space likely requires activation of numerous signaling pathways involving trophic factors, extracellular matrix adhesion proteins and regulators of chemotaxis. For example, tumor cell expression of CD74, a cell surface protein associated with MHC class II, may be one way tumors are attracted to nerves, particularly for pancreatic carcinomas.[49] In OSCC, Kolokythas et al. have shown that expression by tumor cells of nerve growth factor (NGF), a member of the neurotrophin family that is associated with survival and signaling in many neural cell types, and its receptor, receptor tyrosine kinase A (TrkA), is correlated with the development of PNI.[50]

Nerve-specific adhesion complexes and extracellular matrix proteins have been implicated as molecular determinants of PNI as well, with the belief being that expression of these proteins by tumor cells may facilitate cell-substrate interaction, enabling tumor cells to use neural cells and tissues as a conduit for spread.[51] Adhesion molecules not only mediate cell binding but also activate signal transduction pathways associated with morphogenesis in certain physiologic and pathologic conditions. Alterations in cell–cell and cell–matrix adhesions are considered to be important factors in OSCC growth and dissemination.[52] In a study by McLaughlin et al., expression of neural cell adhesion molecule (N-CAM, also known as CD56), a member of the immunoglobulin superfamily of proteins, positively correlated with PNI in OSCC.[51] However, Solares and co-workers could not confirm these findings and found no relationship between N-CAM expression and neurotropism.[53] Other adhesion molecules expressed in OSCC related to histopathological evidence of PNI include ICAM-5 (telencephalin)[52,54] and claudin 1, a component of tight junctions.[55] On the other hand, claudin 4 was associated with a decrease in PNI.[56] An extracellular matrix protein, laminin 5, has been associated with increased PNI in OSCC.[57] Snail, an E-cadherin transcription repressor involved in the acquisition by transformed epithelial cells of a mesenchymal-like phenotype, known as the epithelial to mesenchymal transition, was found to be associated with poor differentiation, basaloid features and lymphovascular invasion, but not PNI.[58] Finally, our group and others have evidence that the plexins and semaphorins, proteins originally shown to be important in nerve cell adhesion, axon migration and proper central nervous system development, are strongly expressed in both axons and many carcinomas, and may play a role in PNI in prostate cancer and OSCC.[59]

Proteins that control cell growth, differentiation and apoptosis also have been identified as correlating with PNI. Chang et al. found that in OSCC tissue there was abundant expression of a member of transforming growth factor-β (TGF-β) superfamily of secreted signaling molecules, activin A, and its inhibitor, follistatin (FST). Their immunohistochemical stain for activin A and FST in 92 patients with OSCC positively correlated with N stage, poor histological differentiation and PNI.[60] Expression of the pro-apoptotic protein Bim/Bod, and the anti-apoptotic BAG-1, members of the Bcl-2 family of apoptosis regulators, was associated with the presence of PNI in an immunohistochemical analysis of OSCC.[61] An analysis performed for p73, a member of the p53 family of tumor suppressors, showed an association with distant metastasis and perineural and vascular invasion.[62] A summary of these findings is shown in Table 1.

Axonogenesis, neurogenesis, and cancer

Emerging models of PNI strongly suggest that interactions between tumor cells and nerves not only induce tumor cell migration but also stimulate axonogenesis, or the enlargement of nerves,

Table 1
Molecular factors exhibiting significant correlation with PNI in OSCC.

Factor	Basic role	Ref.	No. of cases/experiment	Notes
Nerve growth factor (NGF)/tyrosine kinase A (TrkA)	Neurotropic factor	[50]	21/IHC	The authors show a statistically significant correlation of NGF and TrkA expression in the cytoplasm of malignant OSCC cells in tumors with histologic evidence of PNI
Neural cell adhesion molecule (N-CAM)	Neural cell surface glycoprotein belonging to the immunoglobulin superfamily of adhesion molecules	[51]	76/IHC	There is a positive correlation between the presence of N-CAM and PNI in OSCC
		[53]	14/IHC	N-CAM expression did not predict neurotropism in these patients
ICAM-5 (telencephalin)	Adhesion molecule	[52]	25 OSCC specimens and 30 cell lines/ IHC, QRT-PCR, *in vitro* functional assays	Overexpression of ICAM-5 plays a role in OSSC tumorigenesis, possibly through the PI3 K/Akt pathway
Claudin 1	Tight junction protein	[55]	100/IHC, QRTPCR	Claudin 1 overexpression is associated with angiolymphatic and neural invasion, consistent with aggressive tumor behavior
Claudin 4	Tight junction protein	[56]	136/IHC-TMA	Strong expression of claudin 4 was associated with decreased PNI
Laminin 5 (Laminin-332)	Component of basement membrane of skin and mucosa	[57]	64/IHC	Found a significant correlation between staining of laminin 5, an important extracellular matrix protein required for efficient cell motility, and the presence of PNI in OSCC
Activin A	TGF-β family cytokine	[60]	92/IHC	Activin A was correlated with positive N stage, poor histological differentiation, and PNI
Bim/Bod, BAG-1	Bcl-2 family of apoptosis regulators	[61]	229/IHC-TMA	Increased expression of Bim/Bod and BAG-1 was associated with the presence of PNI
p73	Tumor suppressor elonging to the p53 gene family	[62]	38/IHC	p73 expression was associated with distant metastasis and neural and vascular invasion
Snail	Transcription factor important in the epithelial mesenchymal transition during tumor progression	[58]	42/IHC	Snail positive tumors were strongly associated with lymphovascular invasion but not PNI

IHC = immunohistochemistry; TMA = tissue microarray; QRT-PCR = quantitative reverse transcriptase polymerase chain reaction.

axon extension or increased axon number, and neurogenesis, an increase in neuron body cell numbers, that can lead to increased nerve density in and around neurotropic malignancies. This process, important in many normal physiologic processes such as growth, development and wound healing, is a newly recognized phenotype for tumor progression.[59,63,64] Some pathological conditions like Alzheimer's disease and age-related neural degeneration are caused by defects in neurogenesis.[65] This phenomenon may play a role in PNI in adenoid cystic carcinoma, which can express high levels of NGF to attract small peripheral nerve branches to the developing tumor.[66] Ayala and colleagues have been studying this phenomenon in prostate cancer and have concluded that interactions between prostate cancer cells and nerves can create a microenvironment that stimulates both of these cell types to grow towards each other.[59] Whether or not neurogenesis is important for PNI in OSCC and the exact mechanisms explaining how this occurs remain unknown.

Conclusions

Treatment failures in patients with OSCC are primarily due to loco-regional recurrence and distant metastasis. Among different parameters, PNI is a widely accepted clinical and histopathological feature that is frequently associated with aggressive disease and a poor prognosis. However, we did detect variations in the prognostic significance of PNI throughout the literature, probably due to a lack of consistent methodology and study design, a limitation in the number of cases analyzed, and the method of detection of PNI.

Though it represents a distinct third mode of tumor metastasis, along with lymphatic and blood vessel invasion, PNI is not well studied. A lack of experimental models or even an accurate definition for PNI has hindered progress towards understanding the mechanisms of this phenomenon. Here we reported on the best definitions for PNI and summarized the molecular agents that have been reported to promote PNI in OSCC. Further investigations by *in vitro* and *in vivo* studies are needed. With a better understanding of the mechanisms involved, we can develop therapeutic agents to target this form of tumor spread.

Conflict of interest statement

The authors do not have financial or personal relationships with persons or organizations that would influence or bias this work.

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