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Is Perineural Invasion of Head and Neck Squamous Cell Carcinomas Linked to Tobacco Consumption?

Philipp Baumeister, MD[1,2], Christian Welz, MD[3], Christian Jacobi, MD[1], and Maximilian Reiter, MD[1]

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Abstract

Perineural invasion (PNI) is an underrecognized path of cancer spread, and its causes and mechanisms are poorly understood. Recent research indicates a mutual attraction of neuronal and cancer cells, largely dependent on neurotrophic factors and their receptors. Interestingly, the release of neurotrophic factors occurs upon cigarette smoke/nicotine exposure in a dose-dependent manner, and serum levels correlate with current smoking, number of smoking years, and smoking severity. Among cell types capable of neurotrophic factors secretion are lung and oral fibroblasts. In our study of 178 patients with head and neck squamous cell carcinoma, tumors of current and former smokers showed PNI significantly more often than tumors of never smokers. Moreover, PNI was a marker for aggressive tumor growth. Surprisingly, PNI was more significant for survival than p16 status. Our study warrants further research on PNI in head and neck squamous cell carcinoma with special emphasis on the impact of tobacco consumption to identify suitable candidates for therapeutic interventions.

Keywords

perineural invasion, head and neck squamous cell carcinoma, tobacco consumption, neurotrophic factors

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Perineural invasion (PNI) represents a specific path of cancer spread in a variety of solid malignancies, including head and neck squamous cell carcinoma (HNSCC), and is associated with poor prognosis.[1-4] Perineural growth occurs in a discontinuous manner. Cancers spread centripetally toward the central nervous system, forming skip lesions.[5] Thus, PNI-positive (Pn1) HNSCCs may be particularly difficult to resect.[6] PNI also represents a characteristic of more aggressive carcinomas.[7]

Causes and mechanisms of PNI are poorly understood. Recent research indicates that a mutual tropism between pancreatic cancer cells and neurons induces PNI.[8] Deborde et al discovered a subpopulation of Schwann cells in murine and human specimens that associate with pancreatic cancer cells.[9] In cocultures with dorsal root ganglion extracts, these Schwann cells directed cancer cells to migrate toward nerves in a contact-dependent manner. The neural cell adhesion molecule (NCAM), activated by hemophilic binding, was expressed on both Schwann and cancer cells. In their study, cancer cell dispersion and directed invasion of cancer cells were dependent on NCAM expression of Schwann cells. NCAM is expressed by 93% of Pn1-HNSCCs.[10] NCAM is also an alternative receptor of glial cell–derived neurotrophic factor (GDNF), and cancer cell invasion of neuronal structures is partially dependent of GDNF secretion of neuronal cells.[11,12] Furthermore, nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and their respective receptors, tropomyosin-related kinase A and B (TrkA and TrkB), are involved in PNI.[13]

Very recent studies indicated a role of the neurotrophic factors in smoking and the development of nicotine dependence.[14,15] The aim of our study was to investigate possible associations between smoking and PNI in HNSCC.

Methods

We retrospectively analyzed PNI in the HNSCC of patients prospectively enrolled in our department in an ongoing study about risk factors of HNSCC.[16] Patients were interviewed about smoking habits on the day before surgical resection of their tumors. Former smokers were defined as patients who stopped smoking at least 1 year before diagnosis. All patients signed an informed consent regarding the use of clinical data. The study was approved by the Ethics

[1]Department of Otorhinolaryngology–Head and Neck Surgery, Ludwig-Maximilians-University, Munich, Germany
[2]Clinical Cooperation Group—Personalized Radiotherapy in Head and Neck Cancer, Helmholtz Center, Munich, Germany
[3]Department of Otorhinolaryngology–Head and Neck Surgery, Georg-August-University, Göttingen, Germany

Corresponding Author:
Philipp Baumeister, MD, Department of Otorhinolaryngology–Head and Neck Surgery, Ludwig-Maximilians-University, Marchioninistr. 15, Munich, Bavaria 81377, Germany.
Email: Philipp.Baumeister@med.uni-muenchen.de

Committee of the Medical Faculty of Ludwig-Maximilians-University, Munich, Germany. Statistical analysis performed with SPSS 24 (IBM, Chicago, Illinois).

Results

A total of 178 cases were analyzed. Clinical data are shown in **Table 1**: 27.5% (n = 49) of tumors were diagnosed as Pn1 and 72.5% (n = 129) as Pn0. PNI was detected significantly more often in HNSCC of current (34.7%, n = 95) and former (26.1%, n = 46) smokers as compared with never smokers (10.3%, n = 29; P = .036, chi-square test; see **Figure 1**). Spearman correlation between smoking status and PNI was also significant (P = .014).

In the p16-negative group, PNI was found in the tumors of 30.9%, 25.0%, and 0% of current, former, and never smokers, respectively; these percentages were 19.0%, 17.6%, and 4.8%, respectively, in the p16-positive group. Overall, 26.4% of p16-negative and 12.9% of p16-positive HNSCCs were diagnosed as Pn1 (P = .045, chi-square test). Noteworthy, Pn1-HNSCC significantly more often showed additional signs of aggressive growth in terms of lymphovascular invasion (40.0% vs 17.9%, P = .001, chi-square test), vein invasion (64.3% vs 23.0%, P = .001, chi-square test), and extranodal extension (37.3% vs 12.7%, P = .025, chi-square test).

Median follow-up was 2.0 years (range, 0.6-7.0 years). Patients with Pn1-HNSCC had a significantly shorter disease-free survival (3.9 years [95% CI, 2.9-4.9] vs 5.1 years [95% CI, 4.5-5.7], P = .013276, log-rank test) and overall survival (3.0 years [95% CI, 2.2-3.8] vs 5.4 years [95% CI, 4.8-5.9], P = .000004, log-rank test; see **Figure 2**). Surprisingly, patients with p16-positive HNSCC did not have a longer disease-free survival (5.0 years [95% CI, 4.3-5.7] vs 4.1 years [95% CI, 3.6-4.6], P = .170799, log-rank test) within this follow-up period. Overall survival of patients with p16-positive HNSCC was significantly longer (5.8 years [95% CI, 5.2-6.3] vs 3.7 years [95% CI, 3.2-4.3], P = .001297, log-rank test). In multivariate analysis, PNI (P = .000661, Cox regression) was more significant for overall survival when compared with p16 (P = .006185, Cox regression).

Discussion

Our results suggest an association between tobacco consumption and PNI in HNSCC. Furthermore, PNI was related to established characteristics of aggressive tumor growth.

PNI is dependent on the presence of neurotrophic factors.[4] Higher expression levels of NGF and its receptor TrkA were detected in Pn1 versus Pn0 HNSCC.[17] TrkB and GDNF were more expressed in HNSCC tumor specimens than in surrounding mucosa. Blockade of NGF/TrkA signaling decreased proliferation of cancer cells.[18,19] GDNF increased matrix metalloproteinase expression and, thus, migration of oral cancer cells.[20] As reviewed recently, BDNF/TrkB signaling regulates cell migration, invasion, epithelial-to-mesenchymal transition, and cisplatin resistance in HNSCC.[21]

Table 1. Clinical Data.

	n (%)
Localization	
Oral cavity	33 (18.5)
Oropharynx	128 (71.9)
Hypopharynx	13 (7.3)
Larynx	4 (2.2)
Smoking	
Current	29 (16.3)
Former	46 (25.8)
Never	95 (53.4)
N/A	8 (4.5)
T-staging[a]	
pT1	23 (12.9)
pT2	78 (43.8)
pT3	66 (37.1)
pT4a	10 (5.6)
pTx	1 (0.6)
N-staging[a]	
pN0	52 (29.2)
pN1	22 (12.4)
pN2a	16 (9.0)
pN2b	47 (26.4)
pN2c	22 (12.4)
pN3	3 (1.7)
pNx	16 (9.0)
p16	
Positive	62 (34.4)
Negative	87 (48.9)
N/A	29 (16.3)
Perineural invasion	
Pn0 (absent)	129 (72.5)
Pn1 (present)	49 (27.5)
Lymphovascular invasion	
L0 (absent)	106 (59.6)
L1 (present)	70 (39.3)
N/A	2 (1.1)
Vein invasion	
V0 (absent)	161 (90.4)
V1 (present)	14 (7.9)
N/A	3 (1.7)
Extranodal extension	
Positive	51 (28.7)
Negative	51 (28.7)
N0[b]	67 (37.6)
N/A	9 (5.0)
Grading	
G1	4 (2.2)
G2	57 (32.0)
G3	117 (65.7)

Abbreviation: N/A, not available.
[a]*AJCC Cancer Staging Manual*, 7th Edition.
[b]Clinically or pathologically N0.

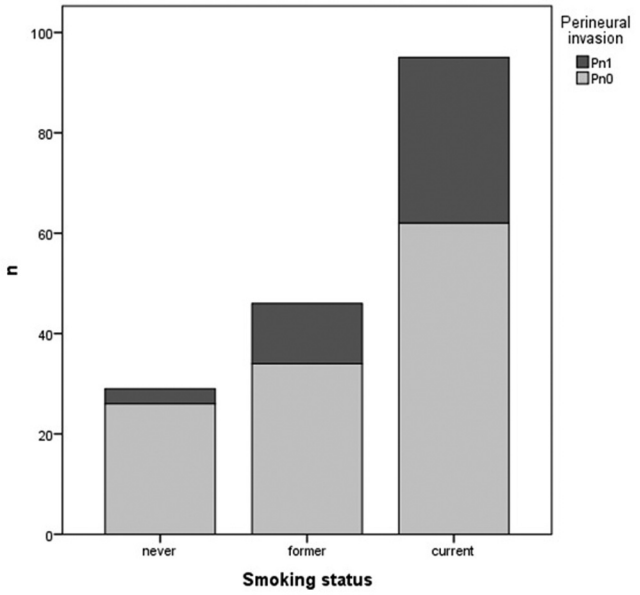

Figure 1. Percentage of perineural invasion–positive carcinomas (Pn1) in relation to smoking status. Pn0, negative.

There is growing evidence of an association between neurotrophic factors and smoking. BDNF influences nicotine dependence. Current smoking and higher number of smoking years were associated with higher BDNF serum levels.[14] BDNF serum levels also correlated with exhaled carbon monoxide and, therefore, smoking severity.[22] BDNF is released from blood platelets exposed to cigarette smoke in a dose-dependent manner.[23] It is secreted by airway smooth muscle cells and lung fibroblasts upon cigarette smoke/nicotine exposure.[24,25] Dudás et al showed that oral fibroblasts are capable of producing BDNF.[26]

In summary, a link among tobacco consumption, neurotrophic factors, and PNI in HNSCC seems plausible, even though other causative factors may play a role. Our results indicate a link between smoking and PNI—to our knowledge for the first time—and so warrant further investigations into proposed connections, not least to identify suitable candidates and targets for therapeutic interventions.

Author Contributions

Philipp Baumeister, acquisition, analysis of data, drafting the work, final approval, accountable for all aspects of the work; **Christian Welz**, acquisition, analysis of data, drafting the work, final approval; **Christian Jacobi**, drafting the work, revising it critically for important intellectual content, final approval; **Maximilian Reiter**, acquisition, analysis of data, drafting the work, final approval, accountable for all aspects of the work.

Disclosures

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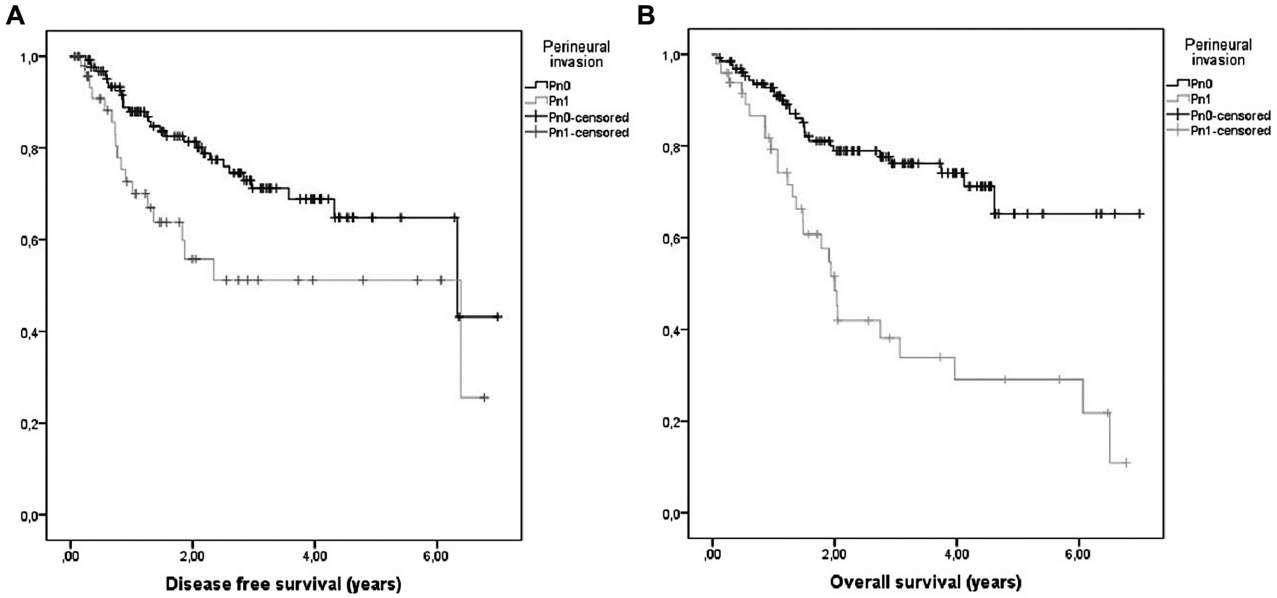

Figure 2. Disease-free survival (A) and overall survival (B) in dependence of perineural invasion. Pn0, negative; Pn1, positive.

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