NEOPLASIA
www.neoplasia.com

Volume 20 Number 7 July 2018 pp. 657–667 657

Redefining Perineural Invasion: Integration of Biology With Clinical Outcome[1]

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Abstract

A diagnosis of perineural invasion (PNI), defined as cancer within or surrounding at least 33% of the nerve, leads to selection of aggressive treatment in squamous cell carcinoma (SCC). Recent mechanistic studies show that cancer and nerves interact prior to physical contact. The purpose of this study was to explore cancer-nerve interactions relative to clinical outcome. Biopsy specimens from 71 patients with oral cavity SCC were stained with hematoxylin and eosin and immunohistochemical (IHC; cytokeratin, S100, GAP43, Tuj1) stains. Using current criteria, PNI detection was increased with IHC. Overall survival (OS) tended to be poor for patients with PNI ($P = .098$). OS was significantly lower for patients with minimum tumor-nerve distance smaller than 5 μm ($P = .011$). The estimated relative death rate decreased as the nerve-tumor distance increased; there was a gradual drop off in death rate from distance equal to zero that stabilized around 500 μm. In PNI-negative patients, nerve diameter was significantly related to OS (HR 2.88, 95%CI[1.11,7.49]). Among PNI-negative nerves, larger nerve-tumor distance and smaller nerve diameter were significantly related to better OS, even when adjusting for T-stage and age (HR 0.82, 95% CI [0.72,0.92]; HR 1.27, 95% CI[1.00,1.62], respectively). GAP43, a marker for neuronal outgrowth, stained less than Tuj1 in nerves at greater distances from tumor (OR 0.76, 95% CI[0.73,0.79]); more GAP43 staining was associated with PNI. Findings from a small group of patients suggest that nerve parameters other than presence of PNI can influence outcome and that current criteria of PNI need to be re-evaluated to integrate recent biological discoveries.

Neoplasia (2018) 20, 657–667

Introduction

Head and neck squamous cell carcinoma (SCC) is the sixth most common cancer in the world with ~600,000 new cases a year [1]. Almost half of these patients will die within 5 years of diagnosis making this the fifth most common cause of cancer-related deaths [2]. SCC has a high incidence of neural invasion [3,4]. Also known as perineural invasion (PNI), this type of invasion is strongly correlated with recurrence and poor survival because nerves are a significant route of tumor spread toward the brain stem and into other nerves [5,6]. Treatment of SCC is currently based on tumor stage, i.e. tumor size, spread to lymph nodes, and spread to distant sites. Detection of PNI enhances the likelihood of lymph node recurrence and leads to selection of radiation and/or elective lymph node dissection as treatment [7,8].

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[1] Conflict of interest: The authors have declared that no conflict of interest exists.

Received 22 February 2018; Revised 18 April 2018; Accepted 18 April 2018

 1476-5586
https://doi.org/10.1016/j.neo.2018.04.005

PNI occurs in multiple cancers; the definition has evolved extensively from the original description of 'tumor growing along nerves' [9] and remains subjective. 'Clinical PNI' also known as 'perineural spread', refers to neural invasion captured by imaging, while PNI represents microscopic, asymptomatic findings [10]. Batsakis (1985) [11] first described PNI in SCC as "invasion in, around and through" nerves. Currently, PNI is defined as "tumor in close proximity to nerve and involving at least 33% of its circumference or tumor cells within any of the 3 layers of the nerve sheath" [12]. This definition includes perineural and intraneural invasion but could vary in interpretation due to terms such as 'close proximity' and the cut-off of 33%. In fact, there is disagreement on the microscopic interpretation of PNI in tissue specimens even among pathologists [13]. The confounding issues and the importance of a diagnosis of PNI in treatment selection highlight the need for establishing an objective, widely acceptable definition for PNI.

Recent evidence supports that PNI is a dynamic process involving mutual tropism between the tumor and nerve. Several groups have shown that nerves and tumor cells communicate prior to physical contact [14–16]. For example, in SCC we showed that galanin released by nerves induces galanin receptor 2 on SCC cells, to promote release of cytokines [14]. In turn, the cytokines promote invasion and neuritogenesis. Importantly, these findings highlight that nerves and cancer are biochemically committed prior to physical contact. As understanding about biologic mechanisms of neural invasion increases, so does the need for an evolving definition of PNI that encompasses different aspects of progression, from early stages when the tumor and nerve are far apart until physical contact and invasion of the nerve sheaths.

In this study, we comprehensively analyzed nerve-tumor interactions in human SCC tissue specimens to investigate correlations with clinical outcome. We observed that among PNI-negative nerves (using current criteria), smaller nerve-tumor distance and larger nerve diameter were significantly associated with worse patient survival, suggesting a role for nerves in SCC aggressiveness. Findings from a small group of patients suggest that nerve parameters other than presence of PNI can influence outcome and that current criteria of PNI need to be re-evaluated to integrate recent biological discoveries into an objective, reproducible, and clinically-relevant definition.

Table 1. Demographic and Disease-Related Characteristics of 71 Patients

Patient Characteristics	N (%)
Gender	
Male	41 (57.7)
Female	30 (42.2)
Age	Years
Mean	60.2
SD	12.9
Tumor Characteristics	**N (%)**
Oral Cavity Subsite	
Gum	15 (21.1)
Mouth Floor	10 (14.1)
Tongue	34 (47.9)
Retromolar area	9 (12.7)
Other	3 (4.2)
T Stage	
1	12 (16.9)
2	21 (29.5)
3	12 (16.9)
4	26 (36.6)
N Stage	
0	41 (57.7)
1	8 (11.2)
2	1 (1.4)
2a	2 (2.8)
2b	17 (23.9)
2c	2 (2.8)
Histopathologic Characteristics	**N (%)**
Differentiation	
Poor	11 (15.4)
Moderate	27 (38.0)
Well	33 (46.4)
Worst Pattern of Invasion (POI) *	
POI 1	5 (7.0)
POI 2	19 (26.7)
POI 3	16 (22.5)
POI 4	26 (36.6)
POI 5	5 (7.0)
PNI (H&E)	
No	55 (77.4)
Yes	16 (22.5)
PNI (H&E + IHC**)	
No	47 (66.2)
Yes	24 (33.8)
Expanded N Positivity (H&E + IHC**)	
N_0, PNI-negative	30 (42.2)
N_0, PNI	11 (15.5)
N-positive	30 (42.2)

* Worst POI assessed as in Brandwein-Gensler et al. 2005.
** PNI assessed using H&E and IHC stains.

Methods

Patient Population

De-identified tissue sections of human SCC were obtained from the Tissue Core of the University of Michigan Head and Neck cancer Specialized Program of Research Excellence (HNSPORE). University of Michigan Institutional Review Board approval and patient consent were obtained by the University of Michigan HNSPORE prior to specimen collection. The study population consisted of 71 patients with oral cavity SCC (41 males and 30 females), with a mean age of 60.2 years. The median follow-up time was 56.2 months. All patients were treated with surgery; 29 (40.8%) patients received surgery alone while 20 (28.2%) patients received adjuvant radiotherapy and 22 (31%) patients received adjuvant chemoradiation. SCC recurred in 18 patients (25.3%) and 14 (19.7%) patients died due to disease. Table 1 summarizes the demographic and disease-related characteristics of the patient population.

Immunohistochemistry

Sections of 5μm thickness were stained with hematoxylin and eosin (H&E) (first and last sections) and sequential sections were stained with the following antibodies (all 1 h, room temperature): S100 purified immunoglobulin fraction of rabbit polyclonal antiserum (Dako, Z0311, 1:1500) to identify nerves, cytokeratin AE1/AE3 mouse monoclonal antibody (EMD Millipore, IHCR2025–6, 1:6) to highlight epithelium, Tuj1 mouse monoclonal antibody (Sigma, T8578, 1:500) to identify β-Tubulin III protein in the axons, and anti-GAP43 rabbit affinity purified polyclonal antibody (Novus Biologicals, NB300–143, 1:2000) to identify regenerating axons and neuronal outgrowth. Mouse (Dako, X0931) or rabbit IgG (Dako, X0936) were used as negative controls at the same concentration as primary antibodies. Antigen retrieval was