Perineural Invasion in Human Cutaneous Squamous Cell Carcinoma Is Linked to Neurotrophins, Epithelial-Mesenchymal Transition, and NCAM1


TO THE EDITOR

Perineural invasion (PNI) in human cutaneous squamous cell carcinoma (SCC) has been recognized since 1984 as being associated with adverse disease-specific survival (Ballantyne, 1984). PNI is also a factor for poor prognosis in other cancers such as head and neck SCC (HNSCC) (Roh et al., 2015) and pancreatic and colorectal cancers (Knijn et al., 2016).

PNI in cutaneous SCC has been recently added to the American Joint Committee on Cancer staging system (Farasat et al., 2011), but so far no study has focused on the mechanisms implicated in PNI in cutaneous SCC.

Here, we studied the mechanisms of PNI on two matched groups of 15 patients with cutaneous SCC, one group with PNI and one without, detected on five different levels in paraffin blocks (see Supplementary Table S1 online). The study was approved by the institutional review board of Hôpital Saint-Louis (Paris, France), and written informed patient consents were obtained according to the Helsinki Declaration. The 5-year follow-up showed larger numbers of recurrences and metastases, and greater disease-specific mortality in patients with PNI. This is in line with a recent risk factor review for cutaneous SCC (Thompson et al., 2016).

The neurotrophins (NGF, BDNF, NT-3, and NT-4) and their receptors (Trk-family and p75NGFR) are the prime candidates for PNI study because of their potent effects on neuronal growth (Liebig et al., 2009). First, we focused on and compared these factors in the 15 cutaneous SCCs with PNI in two different areas, (i) tumor cells distant from the PNI area and (ii) perineural tumor cells. We performed a study combining the analysis of in situ protein expression using immunohistochemistry on full-thickness skin samples and mRNA quantification on laser microdissected tumor cells (see Supplementary Materials and Methods online). This

Abbreviations: EMT, epithelial-mesenchymal transition; HNSCC, head and neck squamous cell carcinoma; PNI, perineural invasion; SACCC, salivary adenoid cystic carcinoma; SC, Schwann cell; SCC, squamous cell carcinoma

Supplementary Material

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.03.1502.

REFERENCES


SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at www.jidonline.org, and at https://doi.org/10.1016/j.jid.2018.03.1502.

REFERENCES


systematic study of surgical pieces enabled us to assess PNI around deep-seated nerves, which can rarely be analyzed on skin biopsy samples, and to perform precise laser microdissection of these deep perineural tumor cells (Figure 1). Using immunohistochemistry, in perineural tumor cells, we found strong expression of BDNF, TrkB, and p75NGFR, contrasting with weak expression of these markers in tumor cells distant from the PNI area. No significant mRNA expression was found for TrkA, TrkC, NGF, NT3, or NT4 (Figure 2a).

Using digital droplet PCR on laser microdissected tumor cells, we quantified mRNA expression levels for neurotrophins and their receptors, and we found significantly higher expression for BDNF, TrkB, and p75NGFR in perineural tumor cells compared with tumor cells distant from the PNI area. No significant mRNA expression was found for TrkA, TrkC, NGF, NT3, or NT4 (Figure 2a).

TrkB (see Supplementary Figure S2 online), a high-affinity receptor for BDNF and NT4, is a potent specific suppressor of anoikis and a metastasis inductor in rat intestinal epithelial cells (Douma et al., 2004). TrkB+/BDNF+ human gastric cancer cells injected into nude mice established peritoneal dissemination, which was suppressed with Trk antagonist K252a (Okugawa et al., 2013). Experimental data also support the therapeutic potential of disrupting Trk signaling events in human prostatic and pancreatic cancers, where PNI is a recognized prognostic factor (Miknyoczki et al., 2002). The TrkB/BDNF pathway plays a pivotal role in tumor cell migration and invasion in HNSCC, which microscopically appear very similar to cutaneous SCC. In an in vitro study, activation of TrkB by BDNF in human HNSCC cell lines induced chemotaxis and invasion, which were both suppressed with a transient knockdown of the TrkB system (Kupferman et al., 2010). In a xenograft of human HNSCC cell lines harboring short hairpin RNA targeting TrkB, tumor growth was suppressed, and an up-regulation of E-cadherin was identified. This link between TrkB and the epithelial-mesenchymal transition (EMT) was also found in vitro in human HNSCC samples and cell lines, where TrkB overexpression was associated with down-regulation of E-cadherin and up-regulation of Twist and Snail1 (Kupferman et al., 2010). In the 15 cutaneous SCCs with PNI studied here, EMT markers were assessed by combining two independent methods (see Supplementary Materials and Methods): immunohistochemistry on tissue sections and digital droplet PCR on laser microdissected tumor cells. Using immunohistochemistry, a decrease in E-cadherin and an increase in Snail1 expression were shown in perineural tumor cells compared with cutaneous SCC samples.
tumor cells distant from PNI areas. There was no difference for vimentin, Slug, Zeb1, Zeb2, Twist1, or Twist2 (Figure 2a, and see Supplementary Figure S1). Using digital droplet PCR, significant E-cadherin down-regulation and Snail1, Slug, Zeb2, and Twist1 overexpression of mRNA were found in perineural tumor cells compared with tumor cells distant from PNI areas. No significant difference was found for vimentin, Zeb1, or Twist2 (Figure 2b). This association between the BDNF/TrkB pathway and EMT has also been shown in epithelial salivary adenoid cystic carcinoma (SACC): in 76 human samples of SACC, BDNF and TrkB overexpression and E-cadherin down-regulation were related to PNI and poor prognosis (Jia et al., 2015). In vitro, co-culture of human SACC and Schwann cell (SC) lines promoted an SC-like differentiation among SACC cells, with down-regulation of E-cadherin and up-regulation of N-cadherin and vimentin. Both SC differentiation and expression of EMT markers were blocked by TrkB antagonist K252a (Shan et al., 2016).

In addition, SCs could also contribute to perineural invasion. In vitro, co-culture of human pancreatic cancer cells with dorsal root ganglion extracts showed that SCs led cancer cells to migrate toward nerves and promoted invasion in a contact-dependent manner. These processes were dependent on NCAM1 expression on SCs and ultimately promoted perineural invasion (Deborde et al., 2016). In our series of 15 cutaneous SCCs with PNI, we observed marked immunostaining of NCAM1 in perineural tumor cells. Conversely, no expression was found for tumor cells distant from PNI (Figure 2a, and see Supplementary Figure S1). Using digital droplet PCR on laser microdissected cells, NCAM1 mRNA was detected in perineural tumor cells but not in tumor cells distant from PNI areas (Figure 2b).

Our results for PNI in cutaneous SCC show processes facilitating perineural tumor cell invasion of the nerve. These data have been confirmed here by the fact that, using immunohistochemistry and digital droplet PCR, no difference across the markers studied was found in the tumor cells distant from nerves between the two groups, with and without PNI.

To our knowledge, we have shown for the first time that PNI in human cutaneous SCC is linked to neurotrophins and the EMT and involves NCAM1, suggesting direct interactions between perineural tumor cells and the nerve.

**CONFLICT OF INTEREST**
The authors state no conflict of interest.

**ACKNOWLEDGMENTS**
Angela Swaine reviewed the English language. The French Society of Dermatology gave financial support.
Sensitivity of Transglutaminase 3 in the IgA Aggregates in Dermatitis Herpetiformis Skin to Potassium Iodide


TO THE EDITOR

Dermatitis herpetiformis (DH), a cutaneous manifestation of celiac disease, is characterized by the deposition of granular IgA in dermal papillary tips. The target antigen of the IgA is transglutaminase 3 (TG3) (Sardy et al., 2002), which co-localizes with the antibody in the deposits. The TG3-specific IgA antibodies are a product of an immune reaction that occurs in the gastrointestinal tract to gluten, a protein found in cereal grains. TG3 is expressed in various cell types in the body, including keratinocytes, where it maintains the integrity of the stratum corneum by connecting epidermal structural proteins (Hitomi, 2005). The IgA-aggregated TG3 retains its activity (Taylor et al., 2015).

In 1978, Plishker et al. (1978) demonstrated that TG purified from human stratum corneum was enzymatically enhanced by trypsin, organic solvents, and chaotropic salts. They found chaotropic agents caused a 10-fold increase in TG activity. The authors speculated the increase in activity was due to conformational change in the enzyme by loss of hydrophobic effect, allowing for increased effectiveness of the active site. This article peaked our interest because a well-known agitator of DH, potassium iodide (KI), is a chaotropic salt.

Since the first reported observation in 1891 that iodine and related compounds can trigger DH (From and Thomsen, 1974), oral or topical iodides were used to diagnose DH, even though its mechanism of action was not known (Alexander, 1975). KI, when applied topically to uninvolved DH skin, will elicit vesicular lesions with perivascular cellular infiltrates similar to those that occur in spontaneously occurring lesions (Reitamo et al., 1981). Lesion progression will vary with the concentration of KI. Clinical testing revealed that 2/21 DH patients had a papular and/or vesicular reaction to a 0.6 M KI topical patch, while 14/21 patients developed a vesicular reaction to the topical KI concentration was increased to 1.8 M (Michaelssson and Svensson, 1975).

We hypothesized that the IgA-aggregated enzyme in DH skin would show a similar increase in activity when subjected to comparable concentrations of KI. The procedure we used in testing for TG3 activity was similar to our earlier reported procedure with slight modifications (Taylor et al.,...