Editorial

Insight into the Latest Concepts of Neurotrophism and Perineural Invasion in Head and Neck Cancer

The hallmark of cancer includes an aggressive growth pattern, higher rate of locoregional recurrence and an increased propensity to disseminate and involve distant structures. It is a common feature for many cancers, especially head and neck malignant tumors, to involve neural structures. The invasion was attributed to be a part of its local aggressive nature. But, several studies have demonstrated certain tumors to show specific affinity toward neural tissues, while sparing other vital tissues in its vicinity. This affinity towards neural structures is termed as neurotrophism.[1] Studies estimating the rate of neural invasion have largely been biased. This is due to the lack of a standard definition and diagnosing criteria for evaluating neural invasion. The terminologies used to denote neural invasion include neurotrophic carcinomatous spread and perineural spread.

Batsakis in 1985 published an article titled 'nerves and neurotrophic carcinomas', wherein he gave a broader definition for perineural invasion (PNI). He defined PNI as tumor cell invasion in, around, and through the nerves.[2] The definition was universally accepted as it standardized the diagnosing criteria for PNI. The major disadvantage of Batsakis definition is that it does not specify which layers are involved by the tumor. Modifying Batsakis definition, Veness emphasized the importance of identifying tumor cells within perineurium to designate PNI.[3] However, again the definition leaves behind those lesions with tumor cells in the collagenous layers of epineurium. Liebig et al, in 2009, advocated that the designation of PNI can be assigned when the tumor cells have involved either one of the three layers of the nerve or has an extensive tumor nerve contact, wherein the tumor cells are closely abutting the epineurium.[1] There was a wide variation as to the minimal percentage of tumor nerve contact required to label PNI. Many authors have proposed that tumor cells must cover a minimal of 33% of the circumference of the nerve, to diagnose PNI. They suggested that less than 33% of tumor nerve contact represents focal abutment and not invasion.[1]

The second debate in PNI was about its mechanism of invasion. It was thought that the tumor cells passed through the lymphatic channels in the inner sanctum of the peripheral nerve. Reina et al and several others have demonstrated the lack of a lymphatic system in the inner layers of the peripheral nerves, thus, ruling out a lymphatic spread to result in PNI.[1,4] The second theory was that nerves were paths of lower resistance, enabling tumor cells to easily penetrate and spread to local and distant sites. Further studies have demonstrated multiple layers of collagen and basement membrane surrounding the nerves; thus, debunking the theory that nerves are routes of low resistance.[1,4]

Ayala et al, in 2001, cultured human prostate cell line with mouse dorsal root ganglia.[1,5] They observed the following: (1) tumor cells migrated along the neurites to reach the ganglion, and (2) the neuritis displayed an outward growth towards the tumor cells. When stromal cells were added to the existing culture, they noticed an increase in the affinity of both the tumor cells and the neuritis to each other. Based on their observation, Ayala et al proposed the involvement of three components—nerve cells, tumor cells and stromal cells in the growth pattern. The growth of the neurites toward the tumor cells were attributed to axonal growth. Axonal growth, in turn depends on various axonal guidance molecules and neurotrophic growth factors. Neurotrophic factors are molecules which enable the tumor cells to acquire proinvasive behavior towards neural elements. Among the neurotrophic factors, the brain-derived neurotrophic factor (BDNF), neurotrophin 4/5 (NT-4/5), neurotrophin 3 (NT-3) and nerve growth factor (NGF) have been shown to have a substantial effect on tumor migration/affinity toward neural tissue.[1,5]

Dalal et al and Ketterer et al have demonstrated an increased expression of neurotrophic factors by both the tumor cells and the neural elements in prostate cancer.[6,7] Though neurotrophins signal the direction of tumor cell migration, proteases like matrix metalloproteinase (MMP) are required to pave the path through the extracellular matrix or nerve sheath. Okada et al observed an increase in the invasive pattern of pancreatic cancer cells following the introduction of exogenous neural growth factor (NGF). They observed that the NGF caused an increased production of MMP-2, which in turn enabled the tumor cells to invade more efficiently. They also noticed that glial cell line-derived neurotrophic factor (GDNF) resulted in an increased MMP-9 expression. Thus, it was established that introduction of neurotrophic factors results in an increased expression of gelatinases (MMP-2, MMP-9), aiding the tumor cells to invade the deeper portion of the host tissue.[6,7]

Several groups of malignant head and neck neoplasms have been associated with PNI. Adenoid cystic carcinoma (AdCC), a malignant salivary gland neoplasm is best known for its affinity for neural tissues. More than 50% of AdCCs are reported to exhibit varying degree of PNI.[8,9] Fagan et al and Tai et al have reported increased locoregional

recurrence and a decreased patient survival rate due to PNI.[10] Trigeminal and facial nerves are most commonly involved nerves in head and neck cancer. Most researchers believe that the involvement of V and VII nerve is due to their wide spread innervations in the head and neck region.[1,3]

The mechanism of PNI in head and neck tumors are similar to those mentioned by Okada et al in prostate cancer.[11] Neurotrophins, like NGF, bind to tropomyosin receptor kinase A (trkA) expressed on the surface of the tumor cells. This leads to the activation of the p44/42 mitogen-associated protein kinase signaling pathway. Kowalski et al in 2002 examined the role of brain-derived neurotrophic factor (BDNF) in the PNI of AdCC,[12] They noticed that in spite of the absence of PNI, all the tissue samples stained positive for BDNF. Correlating the data from several other studies implicating the presence of a higher amount of BDNF at the neural invasion front, they proposed a similar role for BDNF in AdCC. Yilmaz et al observed an increased expression in the tropomyosin receptor kinase B (trkB) on cancer cells in comparison with the surrounding normal mucosa.[13] They illustrated a substantial decrease in the growth of the tumor cells *in vitro*, following the blockage of trkB. Yilmaz et al also demonstrated that inhibiting trkB with a small molecule rendered the tumor cell susceptible to chemotherapy. Kolokythas et al and Ye et al observed that tongue squamous cell carcinoma (OSCC) specimens with PNI expressed NGF to a higher percentage in comparison to a weak/negligible expression in OSCC cases without PNI.[14,15]

Neurotrophin-3 and 4 are over-expressed in pancreatic ductal adenocarcinoma in comparison to normal pancreatic tissue. The role of NT-3 and NT-4 in head and neck squamous cell carcinoma (HNSCC) remains unexplored. The GDNF increases the expression of MMP-9 and MMP-13, which in turn increases the invasiveness and metastatic potential of HNSCC. Edvardsen et al showed that neural cell adhesion molecule (NCAM) increase the rate of tumor cell loss, thereby decreasing tumor aggressiveness.[16] Substance 'P' is shown to promote the outgrowth of neurites towards pancreatic cancer cells when co-cultured with mouse dorsal root ganglion. Its role in HNSCC remains undetermined.[1] Nerve growth factor receptor has been related to the perineural invasion seen in malignant melanoma.[1] Its expression in HNSCC is questionable. Further research of the same is necessary to confirm its potential role.

Head and neck squamous cell carcinoma carries a high PNI incidence rate of about 80%. Several studies have shown that PNI increases the locoregional recurrence rate, and thereby decreases the patient survival rate. The 3-year survival rate of PNI positive mucosal OSCC was 23% as comparison to 49% in stage-matched PNI negative OSCC.[17] The role of many of the neurotrophic agents mentioned above in HNSCC remains unexplored. Studies have shown that blocking the neurotrophins and trk group of receptors have substantially reduced tumor aggressiveness and have increased its susceptibility to chemotherapy. Further studies evaluating large scale HNSCC sample will enable us to create a database for PNI associated neurotropins, which in turn may provide us with potential PNI markers and vital therapeutic targets.

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