

# Effects of Nerve Growth Factor Expression on Perineural Invasion and Worse Prognosis in Early-Stage Cervical Cancer

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To the Editor: Several kinds of malignant tumors show perineural invasion (PNI), which can cause locoregional recurrence and reduce the likelihood of survival after surgery. Our previous study has shown that PNI in cervical cancer is associated with other pathology risk features, and other studies have shown that it is a predictor of poor prognosis.<sup>[1-3]</sup> These findings highlight the importance of clarifying the molecular mechanism of PNI.

Neurotrophins, particularly nerve growth factor (NGF), may facilitate PNI. NGF binds with low affinity to two transmembrane receptors, the tropomyosin-receptor kinase (Trk)-A receptor and the p75 neurotrophin receptor (p75NRT). High expression of NGF and TrkA has been linked to PNI in some types of cancer,<sup>[4,5]</sup> but whether this occurs in cervical cancer is unclear. Here, we investigated whether the expression of NGF and its receptors were linked to PNI in cervical cancer. We also investigated whether such expression was linked to patient prognosis, in which case it might serve as a useful prognostic marker.

We retrospectively analyzed 85 cervical cancer patients who underwent surgical therapy at the Affiliated Tumor Hospital of Guangxi Medical University between September 2011 and August 2014. This study was approved by the Institutional Review Board of the Affiliated Tumor Hospital of Guangxi Medical University. All patients met the following inclusion criteria: (1) their tumor was identified as cervical cancer in Stage IB–IIB according to the International Federation of Gynecology and Obstetrics 2009 staging criteria; and (2) they received radical hysterectomy as well as pelvic lymphadenectomy with or without para-aortic lymph node dissection.

Pathology results were independently examined by two pathologists. Absence or presence of PNI was determined based on S-100 protein staining of cervical and uterine tissue after surgery. PNI was defined as the presence of tumor cells within any of the three layers of the nerve sheath, or as the presence of tumor in proximity to a nerve and involving at least one-third of the nerve's circumference. Data were collected on clinical stage, histology subtype, tumor grade, tumor size, depth of stromal invasion, lymph-vascular space invasion, lymph node metastasis, parametrial invasion, and positive vaginal margin.

Tissue sections (3  $\mu$ m) were prepared from surgical samples and immunostained using the Power Vision Two-Step Kit

(ZSG, Beijing, China) according to the manufacturer's instructions, with primary rabbit polyclonal antibodies (Hengxin Biotech, Nanning, China) against NGF (1:200), TrkA (1:200), and p75NRT (1:150). In parallel, control samples were processed using a rabbit IgG polyclonal antibody (Maixin Biotech, Fuzhou, China). The secondary antibody in immunostaining was horseradish peroxidase-conjugated anti-rabbit antibody (Maixin Biotech). Stained sections were analyzed independently by two investigators blinded to sample details.

Immunohistochemistry staining results were assessed both semi-quantitatively and quantitatively. In the semi-quantitative method, the mean percentage of positive tumor cells was determined under the microscope in at least five areas of the tissue slice at  $\times 400$  original magnification. The presence of  $>50\%$  positive cells was scored as 3; 25–50% as 2; 1–25% as 1; and 0% as 0. Samples with scores of 2 or 3 were considered to show high expression, while samples with scores of 0 or 1 were considered to show low expression. In the quantitative approach, the positive rate (PRA) was calculated as the proportion of a microscope field (original magnification  $\times 100$ ) that appeared positively immunostained under a pathology image analyzer (Leica Dmrq 550, Germany). Ten randomly selected areas were used to calculate the mean PRA for each sample.

All patients were followed-up until death or December 2016, with a median follow-up period of 36 months. No patients were lost to follow-up. Data were collected on recurrence and survival.

Differences in expression of NGF, TrkA, or p75NRT between samples from patients with or without PNI were assessed for significance using the Chi-square test in the case of semi-quantitative analysis or the *t*-test in the case of quantitative analysis. Possible associations between the expression of these three proteins, and clinical characteristics of patients were assessed using the Chi-square test. The ability of NGF or TrkA expression to predict survival was assessed using Kaplan-Meier analysis, and

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the differences were assessed for significance using the log-rank test. All analyses were performed using SPSS 17.0 (IBM, Chicago, IL, USA), with the threshold for statistical significance defined to be  $P < 0.05$ .

Among the 85 patients from whom surgical samples were taken, the average age was  $49.1 \pm 6.7$  years, 23 (27%) were PNI-positive, and 62 (73%) were PNI-negative. The staging of patients was as follows: Stage IB1, 20 patients (23.5%); Stage IB2, 27 (31.8%); Stage IIA, 22 (25.9%); and Stage IIB, 16 (18.8%). Most patients (67 [78.8%]) had squamous cell carcinoma while 18 (21.2%) had adenocarcinoma or adenosquamous carcinoma. Only over half (45 [2.9%]) had tumors smaller than 4 cm, while 40 (47.1%) had tumors 4 cm or larger. Tumor grade distribution was as follows: Grade G1, 12 patients (14.1%); Grade G2, 25 (29.4%); and Grade G3, 48 (56.5%). Only under half (36, 42.4%) showed the depth of invasion  $<1/2$  while 49 (57.6%) showed the depth of invasion  $\geq 1/2$ . Only over half (46 [54.1%]) showed invasion of the lymphovascular space, and a slightly higher proportion (50 [58.8%]) showed lymph node metastasis. A small number of patients (11 [12.9%]) showed a parametrical invasion, and even a lower proportion (five [5.9%]) showed a positive vaginal margin. On the basis of postoperative pathology results, 49 patients were given postoperative adjuvant radio- or chemoradiotherapy, of whom 13 were in Stage IB2, 20 in Stage IIA, and 16 in Stage IIB.

Cervical cancer is shown in Figure 1a, where nerve tissue appears dark brown. NGF and TrkA staining localized mainly to the cytoplasm and cytomembrane of cancer cells [Figure 1b]. Of the 85 specimens, 28 were assigned a score of 2 for NGF expression, and 25 were assigned a score of 3. Similarly, 27 specimens received a score of 2 for TrkA expression, and 20 were assigned a score of 3. In contrast, only 12 samples received a score of 2 for p75NRT expression. Staining for p75NRT localized mainly to basal tumor cells [Figure 1b]. High expression (scored as 2–3) occurred in 53 patients (62%) for NGF, 47 (55%) for TrkA, and 12 (14%) for p75NRT.

Semi-quantitation of immunostaining results indicated that high NGF expression was observed in 19 patients (82%) with PNI, compared to 34 (55%) without PNI. The corresponding results for TrkA were 18 (78%) and 29 (16%). These results indicated that the expression of both proteins was significantly associated with PNI ( $P = 0.019$  and  $0.009$ , respectively). In contrast, high p75NRT expression was observed in only five patients (22%) with PNI, compared to seven (11%) without PNI. This indicated no significant correlation between p75NRT expression and PNI ( $P = 0.380$ ). Quantitation of immunostaining results indicated that PRA was significantly higher among samples with PNI than among those without invasion in the case of NGF ( $P = 0.021$ ) and TrkA ( $P = 0.017$ ). In contrast, PRA for p75NRT was similar between the two groups ( $P = 0.365$ ).

Analysis of potential correlations between immunostain-assessed expression and patient clinicopathological characteristics showed that only lymph-vascular space invasion was significantly associated with high expression of NGF ( $P = 0.011$ ) or TrkA ( $P = 0.017$ ). None of the clinicopathological parameters showed an association with p75NRT expression.

Patients were classified as having high or low expression of NGF or TrkA, and their disease-free survival (DFS) and overall survival (OS) curves were compared [Figure 1c]. NGF expression correlated significantly with OS ( $P = 0.040$ ) and DFS ( $P = 0.037$ ). Similar results were observed for TrkA expression with OS ( $P = 0.033$ ) and DFS ( $P = 0.023$ ).

Here, we provide evidence that the expression of NGF and its receptor TrkA is associated with PNI in early-stage cervical cancer. This immediately suggests a testable hypothesis about how NGF may contribute to invasion since the interaction of NGF with TrkA is known to trigger signaling pathways involving PI3-Akt and Ras-MAP, which activate neuronal growth and survival. Our data do not indicate an association of p75NRT expression with PNI or other clinicopathological features of cervical cancer patients. NGF binding to p75NRT activates pro-apoptotic signaling through pathways involving nuclear factor-B and c-Jun N-terminal kinase.<sup>[6]</sup> Therefore, our results imply that NGF might play a positive, stimulatory role in PNI in cervical cancer.

Our results with cervical cancer extend existing understanding on high NGF and TrkA expression in adenoid cystic carcinoma,<sup>[5]</sup> oral squamous cell carcinoma,<sup>[4]</sup> and pancreatic cancer.<sup>[7]</sup> In adenoid cystic carcinoma, p75NRT was expressed only at low level,<sup>[5]</sup> which is consistent with our results. This leads to a general hypothesis that in all these cancer types, NGF stimulates cancer cell proliferation that goes hand-in-hand with downregulation of p75NRT. Our data further suggest that high NGF and TrkA expression, but not p75NRT expression, is associated with PNI in cervical cancer. This extends previous reports linking NGF and TrkA expression to PNI in adenoid cystic carcinoma,<sup>[5]</sup> oral carcinoma,<sup>[8]</sup> and pancreatic cancer.<sup>[7]</sup> These results imply that the proliferative effects of NGF and concomitant downregulation of pro-apoptotic p75NRT help drive PNI.

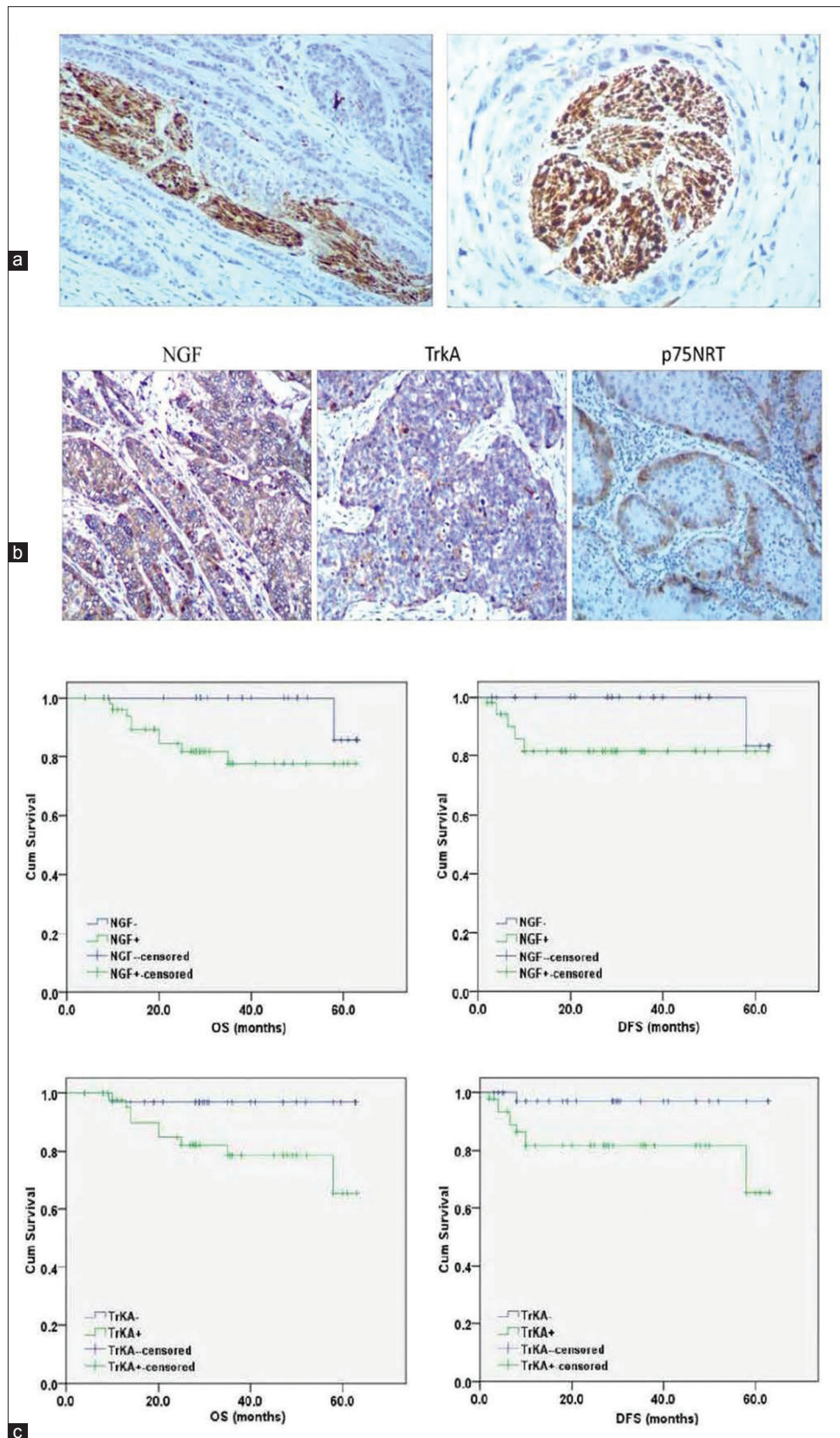
We found that NGF and TrkA expression correlated with the presence of lymph-vascular space invasion, which is a well-known risk factor for cervical cancer recurrence and poor survival and has previously been associated with PNI.<sup>[9]</sup> Lymph-vascular space invasion and PNI are promoted by tumor cell proliferation, invasion, and migration. For example, NGF stimulates the growth of pancreatic tumor cells,<sup>[10]</sup> while blocking NGF signaling in such cells reduces their PNI potential.<sup>[11]</sup> Therefore, we hypothesize that NGF and/or TrkA might have potential as biomarkers of at least certain aspects of malignant tumor behavior. Future work should examine this question in greater detail.

Some studies support the hypothesis that NGF expression level can predict prognosis in cancer,<sup>[5,7,8]</sup> but other work contradicts this idea.<sup>[12]</sup> The ability of NGF and TrkA expression to serve as a marker of tumor prognosis may depend on tumor type. In this study, we showed an association of NGF and TrkA expression with PNI, as well as with DFS and OS. These results add to the argument that NGF and TrkA expression may be useful as prognostic markers in cervical cancer.

In conclusion, our results implicate the expression of NGF and its receptor TrkA in cervical cancer and specifically in PNI. In contrast, the expression of p75NRT does not appear to correlate with PNI. These findings suggest that the proliferative effects of NGF help drive PNI in cervical cancer and that this process involves the downregulation of the pro-apoptotic NGF receptor p75NRT. Analogous to the association of NGF and TrkA with PNI, which strongly affects patient prognosis, we observed an association of NGF and TrkA expression with patient survival. These results provide preliminary evidence that NGF and TrkA might be potential prognostic markers of cervical cancer. Our results should be verified and extended in larger studies focusing on NGF-based mechanisms of PNI.

### Declaration of patient consent

The authors certify that they have obtained informed consent from patients for their clinical information and images to be published.



**Figure 1:** Typical positive immunostaining of PNI in cervical cancer (a). Typical positive immunostaining of NGF, TrkA, and p75NRT in sections of cervical tumors (original magnification  $\times 400$ ; b). Kaplan-Meier survival curves of DFS and OS in patients showing high NGF expression (NGF+), low NFG expression (NGF-), high TrkA expression (TrkA+), or low TrkA expression (TrkA-) (c). PNI: Perineural invasion; NGF: Nerve growth factor; DFS: Disease-free survival; OS: Overall survival; Trk: Tropomyosin receptor kinase.



The patients were told that their name and initials would not be published and that reasonable efforts would be made to conceal their identity, even if absolute anonymity could not be guaranteed.

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Nil.

### Conflicts of interest

There are no conflicts of interest.

### REFERENCES

1. Wei YS, Yao DS, Long Y. Evaluation of the association between perineural invasion and clinical and histopathological features of cervical cancer. *Mol Clin Oncol* 2016;5:307-11. doi: 10.3892/mco.2016.941.
2. Memarzadeh S, Natarajan S, Dandade DP, Ostrzega N, Saber PA, Busuttil A, *et al.* Lymphovascular and perineural invasion in the parametria: A prognostic factor for early-stage cervical cancer. *Obstet Gynecol* 2003;102:612-9. doi: 10.1097/00006250-200309000-00034.
3. Cui L, Shi Y, Zhang GN. Perineural invasion as a prognostic factor for cervical cancer: A systematic review and meta-analysis. *Arch Gynecol Obstet* 2015;292:13-9. doi: 10.1007/s00404-015-3627-z.
4. Kolokythas A, Cox DP, Dekker N, Schmidt BL. Nerve growth factor and tyrosine kinase A receptor in oral squamous cell carcinoma: Is there an association with perineural invasion? *J Oral Maxillofac Surg* 2010;68:1290-5. doi: 10.1016/j.joms.2010.01.006.
5. Kobayashi K, Ando M, Saito Y, Kondo K, Omura G, Shinozaki-Ushiku A, *et al.* Nerve growth factor signals as possible pathogenic biomarkers for perineural invasion in adenoid cystic carcinoma. *Otolaryngol Head Neck Surg* 2015;153:218-24. doi: 10.1177/0194599815584762.
6. Molloy NH, Read DE, Gorman AM. Nerve growth factor in cancer cell death and survival. *Cancers (Basel)* 2011;3:510-30. doi: 10.3390/cancers3010510.
7. Dang C, Zhang Y, Ma Q, Shimahara Y. Expression of nerve growth factor receptors is correlated with progression and prognosis of human pancreatic cancer. *J Gastroenterol Hepatol* 2006;21:850-8. doi: 10.1111/j.1440-1746.2006.04074.x.
8. Yu Yu EH, Lui MT, Tu HF, Wu CH, Lo WL, Yang CC, *et al.* Oral carcinoma with perineural invasion has higher nerve growth factor expression and worse prognosis. *Oral Dis* 2014;20:268-74. doi: 10.1111/odi.12101.
9. Elsahwi KS, Barber E, Illuzzi J, Buza N, Ratner E, Silasi DA, *et al.* The significance of perineural invasion in early-stage cervical cancer. *Gynecol Oncol* 2011;123:561-4. doi: 10.1016/j.ygyno.2011.08.028.
10. Xin B, He X, Wang J, Cai J, Wei W, Zhang T, *et al.* Nerve growth factor regulates CD133 function to promote tumor cell migration and invasion via activating ERK1/2 signaling in pancreatic cancer. *Pancreatol* 2016;16:1005-14. doi: 10.1016/j.pan.2016.09.005.
11. Bapat AA, Munoz RM, Von Hoff DD, Han H. Blocking nerve growth factor signaling reduces the neural invasion potential of pancreatic cancer cells. *PLoS One* 2016;11:e0165586. doi: 10.1371/journal.pone.0165586.
12. Urabe K, Murakami Y, Kondo N, Uemura K, Hashimoto Y, Nakagawa N, *et al.* Nerve growth factor expression is not associated with perineural invasion in extrahepatic cholangiocarcinoma. *Dig Dis Sci* 2016;61:774-84. doi: 10.1007/s10620-015-3953-9.