Translational Oncology
www.transonc.com

Volume 11 Number 3 June 2018 pp. 764–770 764

Cytoplasmic TrkA Expression as a Screen for Detecting *NTRK1* Fusions in Colorectal Cancer[1,2]

Yuri Choi[*], Yeo-Jin Won[*], Sojeong Lee[*], Ahrong Kim[*], Younggeum Kim[*], Won-Young Park[*], Hong-Jae Jo[†], Geun Am Song[‡], Chae Hwa Kwon[*,3] and Do Youn Park[*,3]

[*]Department of Pathology, Pusan National University Hospital and Pusan National University School of Medicine, and Biomedical Research Institute, Pusan National University Hospital, 179 Gudeok-ro, Seo-Gu, Busan 49241, Republic of Korea; [†]Department of Surgery, Pusan National University Hospital and Pusan National University School of Medicine, and Biomedical Research Institute, Pusan National University Hospital, 179 Gudeok-ro, Seo-Gu, Busan 49241, Republic of Korea; [‡]Department of Internal Medicine, Pusan National University Hospital and Pusan National University School of Medicine, and Biomedical Research Institute, Pusan National University Hospital, 179 Gudeok-ro, Seo-Gu, Busan 49241, Republic of Korea

Abstract

NTRK1 gene fusions, the targets of multikinase inhibitors, are promising therapeutic targets for colorectal cancer (CRC). However, screening methods for detecting *NTRK1* gene fusions in CRC tissues have not been reported. In this study, we investigated the potential use of immunohistochemistry (IHC) for detecting *NTRK1* gene fusions. We performed and compared IHC with fluorescence in situ hybridization (FISH) in 80 CRC patients. TrkA immunostaining was observed to be both membranous and cytoplasmic and was scored semiquantitatively using staining intensity and proportions. The tumors were observed to be *NTRK1* gene fusion-positive when ≥20 out of 100 nuclei in FISH. A significant correlation between the IHC and FISH results for determination of the *NTRK1* gene fusions was observed. We measured the cytoplasmic TrkA expression, which showed an area under the receiver operating characteristic (ROC) curve of 0.926 (range: 0.864-0.987, 95% CI, $P = .001$). By choosing 4.5 (sum of the intensity and proportion scores of cytoplasmic TrkA expression) as the cut-off value for the positive and negative *NTRK1* gene fusion groups, the sensitivity and specificity for predicting lymph node metastasis were 100 and 83.8%, respectively ($P = .001$). Specifically, high cytoplasmic TrkA expression (sum of intensity and proportion scores >4) was associated with the presence of *NTRK1* gene fusions ($P < .0001$, $r = 0.528$). Taken together, our data showed that IHC for TrkA can be used as an efficient screening method for detecting *NTRK1* gene fusions in CRC.

Translational Oncology (2018) 11, 764–770

Address all correspondence to: Do Youn Park, MD, PhD, Department of Pathology, Pusan National University Hospital and Pusan National University School of Medicine, 179 Gudeok-ro, Seo-Gu, Busan 49241, Republic of Korea. E-mail: pdy220@pusan.ac.kr

[1] Funding: This work was supported by the National Research Foundation of Korea (NRF) funded by the Korean government (MSIP) (grant no. 2014R1A2A1A11052217). Also, this study was supported by a grant from the National Research and Development Program for Cancer Control, Ministry for Health, Welfare and Family Affairs, Republic of Korea (grant no. 0920050). The biospecimens for this study were generously provided by the Pusan National University Hospital and National Biobank of Korea, which is supported by the Ministry of Health, Welfare, and Family Affairs.

[2] Conflicts of Interest: The authors have declared that no conflicting interests exist.
[3] CH Kwon and DY Park share senior authorship.
Received 2 February 2018; Revised 28 March 2018; Accepted 28 March 2018

1936-5233/18
https://doi.org/10.1016/j.tranon.2018.03.011

Introduction

Colorectal cancer (CRC) is a major cause of morbidity and mortality worldwide. The incidence of CRC in Korea has increased dramatically over the past few decades; however, the incidence rates of other more common cancers, such as stomach and liver cancers, have decreased [1]. Recently, molecularly targeted drugs, such as cetuximab (Erbitux®; Merck KGaA, Darmstadt, Germany), an epidermal growth factor receptor (EGFR)-targeted monoclonal antibody, and the vascular endothelial growth factor (VEGF)-targeted monoclonal antibody bevacizumab (Avastin®; Genentech Inc., CA, U.S.) [2–4], have been developed to treat metastatic CRC. However, serious limitations are associated with the use of these targeted drugs, and it is thus necessary to develop prognostic biomarkers and novel therapeutic targets based on the molecular mechanisms underlying CRC pathogenesis. Recently, due to the development of next generation sequencing (NGS) technology, several studies have revealed candidate therapeutic targets for treating colon cancer, including fusion genes and oncogenic driver mutations [5,6]. Among these, gene fusions with neurotrophic tyrosine kinase receptor 1 (*NTRK1*), such as *TPM3-NTRK1* and *LMNA-NTRK1*, have been reported in CRC [7–10]. *NTRK1* encodes the TrkA receptor, which is a member of the Trk (tropomyosin receptor kinase) family of receptor tyrosine kinases (RTKs) [11]. *NTRK1* activation induces the PI3K/AKT, Ras/MAPK, and PLC-gamma signaling pathways [12]. *NTRK1* gene fusions are therapeutic targets for multikinase inhibitors, such TrkA inhibitors.

Although the optimal method for detecting NTRK1 gene fusions has not yet been determined, three methods, DNA-based NGS assays, targeted panels using RNA, and fluorescence in situ hybridization (FISH) are widely used [13]. DNA-based NGS methods can fail to detect fusion genes due to the analysis of introns (size limitation) and problems with the degradation of using formalin-fixed paraffin embedded tissue (FFPE) specimens. Targeted RNA methods have similar sample quality problems (RNA quality in FFPE tissues). The FISH method has an advantage as it can detect fusions genes in FFPE specimens and has been used in lung cancer [14]; however, the results cannot be used to identify the fusion partner or differences between fusion variants. These methods are time consuming, expensive and have issues with specimen quality. Recently, immunohistochemical (IHC) analysis using an antibody targeted against the C-terminal intracellular domain of TrkA was reported for detecting *NTRK1* gene fusions [8,12]. However, standardization of IHC results for determining TrkA protein expression, similar to the standardization of ALK expression for detecting *ALK* fusion genes in lung cancer [15], has not been reported. Thus, we validated IHC TrkA expression as a screening method for detecting *NTRK1* fusions in 80 CRC tissue samples.

Materials and Methods

Commercially available split FISH probes were used for detecting the *NTRK1* gene fusions in CRC tissues according to the manufacturer's instructions (FS0024; Abnova, Taipei City, Taiwan). The study included a cohort of 80 patients with T3 or T4 CRC, who underwent resection of the primary tumor at Pusan National University Hospital (PNUH) between January and May 2015. The group consisted of 44 men and 36 women, with a mean age of 67.8 years (range, 31-87 years). Standard FFPE sections were obtained from the Department of Pathology and the National Biobank of Korea, Pusan National University Hospital. All samples from the National Biobank of Korea were procured after obtaining informed consent of the patients and were subjected to institutional review board-approved protocols. Briefly, TrkA expression was confirmed in deparaffinized, protease-treated FFPE tissue sections using an anti-TrkA C-terminal monoclonal antibody (TA806413; OriGene, Rockville, MD, U.S.). Brain ganglions and lymphocytes served as positive and negative controls, respectively. TrkA immunostaining was observed to be both membranous and cytoplasmic (Figure 1) and was semiquantitatively scored [staining intensity: negative (0), weak (1), moderate (2), strong (3); staining percentage: 0%-5% (0), 5%-25% (1), 25%-50% (2), ≥50% (3)]. For interpretation of TrkA cytoplasmic and membranous staining, each pathologist (SJL, AK, and DYP) first separately scored and in cases with differing results, samples were reviewed again using a multi-viewer microscope. Simultaneously, deparaffinized and protease-treated FFPE tissue sections were denatured at 75 °C and incubated overnight with 5′-end TexRed-labeled and 3′-end FITC-labeled probes for *NTRK1*. After washing and DAPI counterstaining (32–804,831; Abbott, Chicago, IL, U.S.), the number and localization of hybridization signals were assessed. Tumors were determined to be *NTRK1* gene fusion positive when more than 20 out of 100 nuclei demonstrated break-apart 5′- and 3′-end signals (Figure 2). The relationship between the *NTRK1* FISH-positive and TrkA IHC-positive results was assessed using the Spearman's rank correlation coefficient. The ability of TrkA IHC to predict the detection of *NTRK1* gene fusions by FISH was assessed using ROC curve analysis. Clinicopathological features were analyzed for the *NTRK1* gene fusions using the Student's *t*-test, χ^2 test, or Fisher's exact test. The results were considered to be statistically significant at $P < .05$. Statistical analysis was performed using SSPS version 10.0 for Windows (SPSS Inc., Chicago, IL, USA).

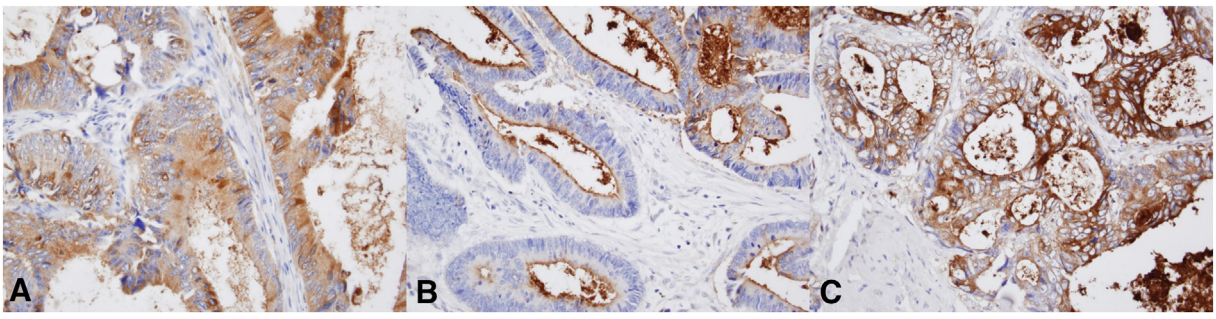

Figure 1. IHC staining patterns of TrkA in CRC tissue samples. (A) Cytoplasmic, (B) membranous, and (C) mixed cytoplasmic and membranous staining patterns are shown.

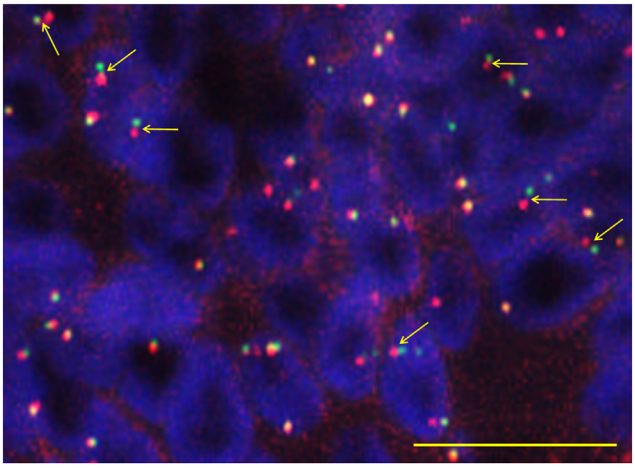

Figure 2. FISH assay using split FISH probes for confirming *NTRK1* rearrangements. Split red and green signals were observed (yellow arrows), which indicated *NTRK1* gene fusions. The scale bar indicates 20 μm.

Results

We detected the expression of *NTRK1* gene fusions and TrkA and determined the relationship between FISH and IHC results for *NTRK1* gene fusion analysis. We simultaneously performed FISH using split FISH probes and IHC using an anti-TrkA C-terminal monoclonal antibody using FFPE sections obtained from 80 CRC patients. The IHC analysis of TrkA in CRC tissue samples revealed two staining patterns (cytoplasmic and membranous). About 80% of the CRC samples (64/80) showed both cytoplasmic and membranous staining. Additionally, heterogeneous cytoplasmic or membranous staining in the CRC tissue sections was observed. Figure 3 shows heterogeneous cytoplasmic staining of adenocarcinoma tissue, which coincides with FISH-positive *NTRK1* gene fusion sites and increased cytoplasmic staining of TrkA.

For the IHC analysis using an anti-TrkA C-terminal monoclonal antibody, we independently evaluated and scored cytoplasmic and membranous TrkA expression. For the intensity scoring of cytoplasmic TrkA expression, negative (0), weak (1), moderate (2), and strong (3) cytoplasmic TrkA expression was noted in 20.0% (16/80), 26.3% (21/80), 38.8% (31/80), and 15.0% (12/80) of samples, respectively. With regard to the proportional scoring of cytoplasmic TrkA expression, 0%-5% (0), 5%-25% (1), 25%-50% (2), and ≥50% (3) cytoplasmic TrkA expression was identified in 21.3% (17/80), 21.3% (17/80), 36.3% (29/80), and 21.3% (17/80) of the samples, respectively. For the intensity scoring of membranous TrkA expression, negative (0), weak (1), moderate (2), and strong (3) membranous TrkA expression was noted in 6.3% (5/80), 40.0% (32/80), 50.0% (40/80), and 3.8% (3/80) of samples, respectively. With regard to the proportional scoring of membranous TrkA expression, 0%-5% (0), 5%-25% (1), 25%-50% (2), and ≥50% (3) membranous TrkA expression was identified in 6.3% (5/80), 11.3% (9/80), 38.8% (31/80), and 43.8% (35/80) of samples, respectively (Figure 3). The *NTRK1* gene fusion analysis using FISH indicated *NTRK1* gene fusions were present in 7.5% (6/80) of the samples. Comparing the results of the two assays for each sample, we observed heterogeneous TrkA immunostaining was associated with either strong cytoplasmic TrkA expression and frequent *NTRK1* rearrangements or weak to moderate cytoplasmic TrkA expression and less frequent *NTRK1* rearrangements (Figure 4).

A significant correlation between the FISH and IHC results for *NTRK1* gene fusion analysis was seen. We observed cytoplasmic TrkA expression with the area under the receiver operating characteristic (ROC) curve as 0.926 (0.864-0.987, 95% CI, P = .001) (Figure 5). By choosing 4.5 (the sum of the cytoplasmic TrkA expression scores) as the cut-off value for the *NTRK1* gene fusion positive and negative groups, the sensitivity and specificity of predictions of lymph node metastasis were 100 and 83.8%, respectively (P = .001). Specifically, high (or strong) cytoplasmic TrkA expression (sum of the intensity and proportion score > 4) was associated with the detection of *NTRK1* gene fusions (P < .0001, r = 0.528) (Table 1). In order to assess the characteristics of the *NTRK1* gene fusion-positive CRC samples, we analyzed various clinicopathological features in all 80 CRC patients. We observed that the *NTRK1* gene fusion-positive tumors were associated with younger patients (P = .051). Other clinicopathological features including sex, tumor location, histological differentiation, perineural invasion, invasion depth, lymphovascular invasion, lymph node metastasis, and microsatellite instability status were not associated with the detection of *NTRK1* gene fusions (Table 2).

Discussion

With the recent development of multi-omics data analysis tools and sequencing methods, several studies have revealed novel therapeutic targets such as gene fusions (e.g., the *NTRK1* and *RSOP* gene fusion in CRC) [5,6]. Among these, the *NTRK1* gene fusions, such as *TPM3-NTRK1* and *LMNA-NTRK1*, have been reported in CRC patients [7–10]. *NTRK1* gene fusions are therapeutic targets for multikinase inhibitors, such TrkA inhibitors. *NTRK1* encodes the TrkA receptor, which is a member of the Trk family of RTKs. Its activation induces the PI3K/AKT, Ras/MAPK, and PLC-gamma signaling pathways [11,12]. The dysregulation of the kinase activity of Trk is associated with carcinogenesis.

We identified *NTRK1* gene fusions in 7.5% (6/80) of CRC samples using FISH. This incidence rate is higher than that reported by previous studies [2.7% (2/74, using NGS), 1.7% (1/66, using qRT-PCR), 2% (3/147, using RNA sequencing), and 0.5% (2/408, using IHC and qRT-PCR)] [7–10]. We believe that these discrepancies can be partly attributed to the differences in detection methods or study cohorts. Unlike other studies, we simultaneously performed FISH and IHC, using split FISH probes using an anti-TrkA monoclonal antibody, in FFPE sections obtained from 80 CRC patients. Lee et al. performed IHC for TrkA and confirmed *NTRK1* gene fusions using NGS [9]. Ardini et al. used qRT-PCR to detect the high expression level of mRNA coding for the intracellular domains of *NTRK1* and confirmed these results using PCR and direct DNA sequencing [7]. Park et al. initially performed RNA-sequencing analysis and then confirmed *NTRK1* gene fusions using FISH [10]. Créancier et al. used IHC and qRT-PCR to detect *NTRK1* gene fusions in CRC samples [8]. However, these methods could potentially yield false-negative and false-positive results. Although testing of fusion genes was based on FISH in many laboratories, FISH and other molecular methods have various limitations (cost, turn-around time, and FFPE specimen quality problems). Conklin et al. reported that IHC was a reliable method to detect *ALK* rearrangements identified by FISH in non-small cell lung cancer [14], and our previous data and studies have shown that strong cytoplasmic TrkA expression was associated with *NTRK1* gene fusions [7–10]. Based on these data and findings, we performed a correlation analysis between

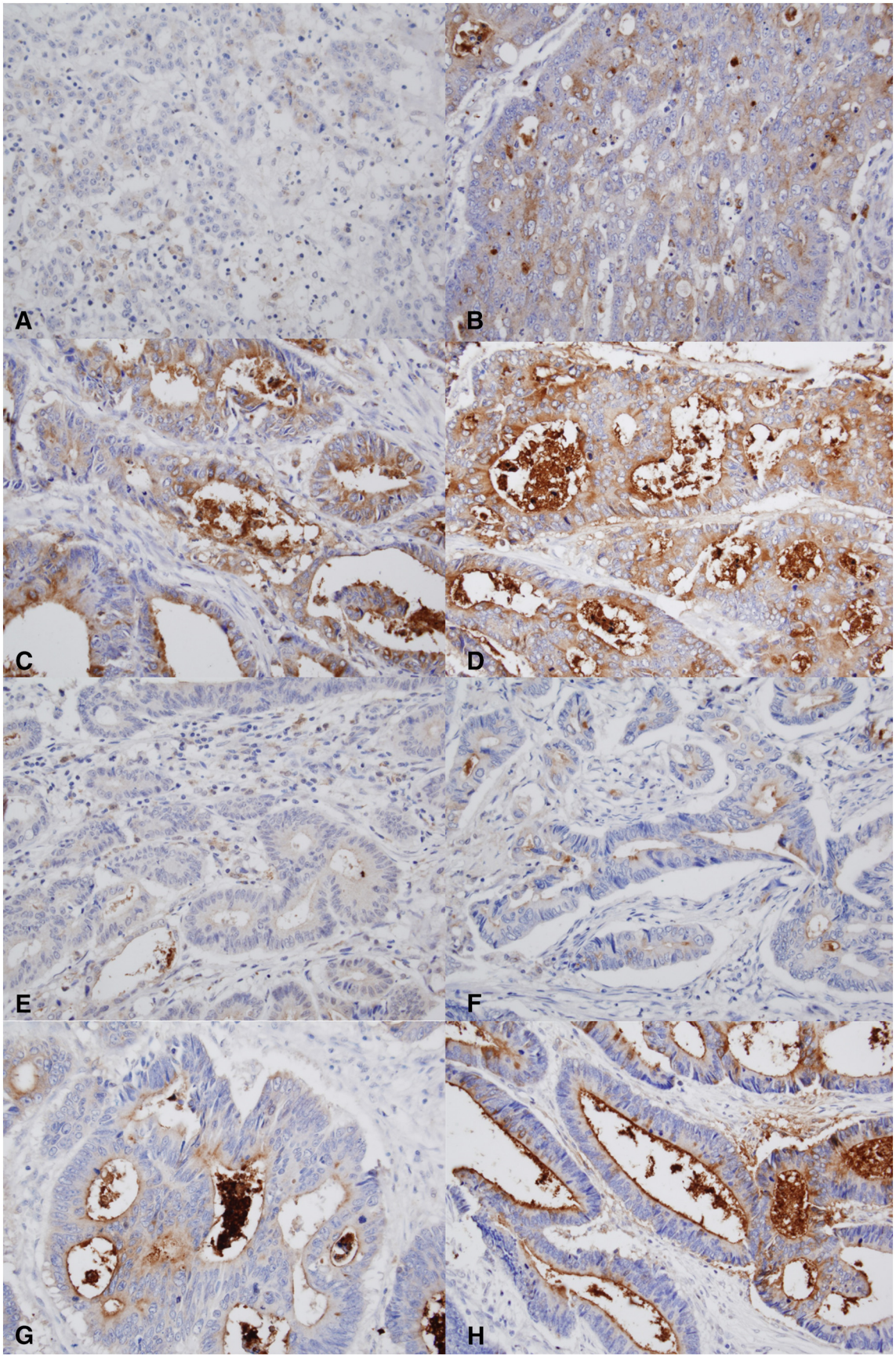

Figure 3. Cytoplasmic TrkA immunostaining showing (A) negative, (B) weak, (C) moderate, and (D) strong staining intensities. Membranous TrkA immunostaining showing (E) negative, (F) weak, (G) moderate, and (H) strong staining intensities.

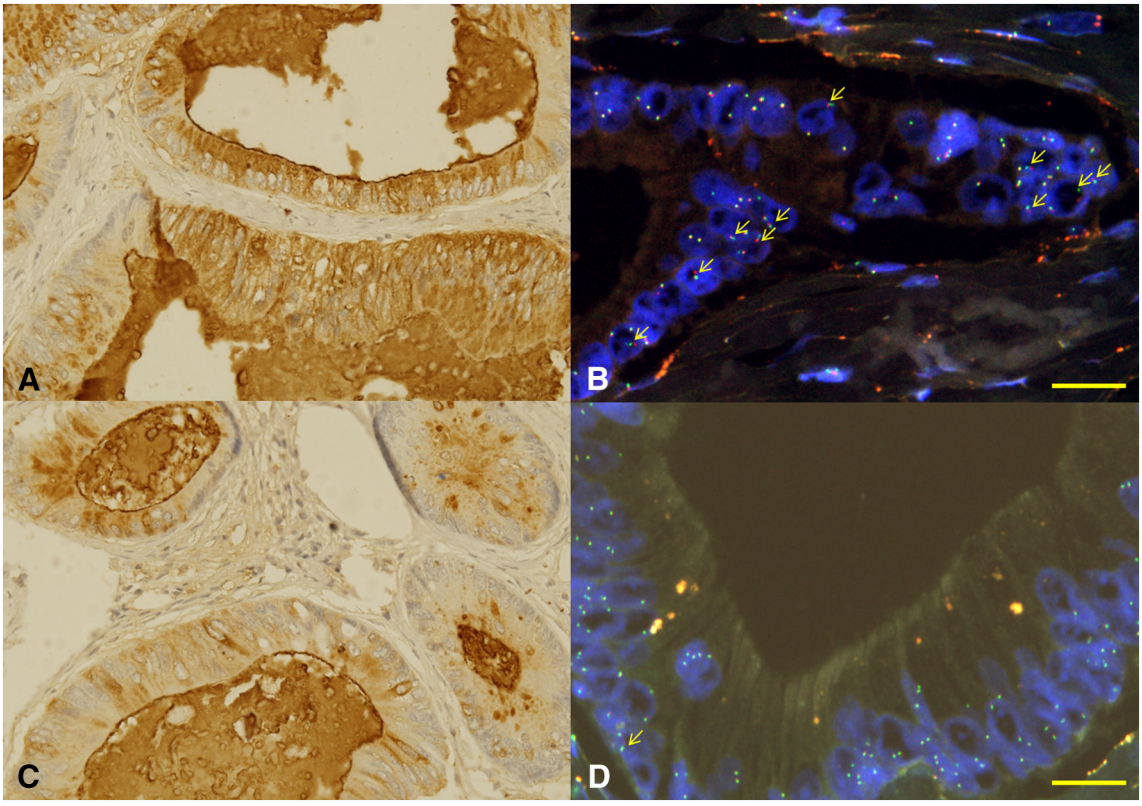

Figure 4. Heterogeneous TrkA immunostaining in the CRC tissues analyzed using FISH. (A & B) Strong cytoplasmic TrkA expression in adenocarcinoma tissues showing frequent *NTRK1* rearrangements. (C & D) Weak to moderate cytoplasmic TrkA expression in adenocarcinoma tissues revealing less frequent *NTRK1* rearrangements. Split red and green signals (yellow arrows) indicate *NTRK1* fusion genes. The scale bar indicates 20 μm.

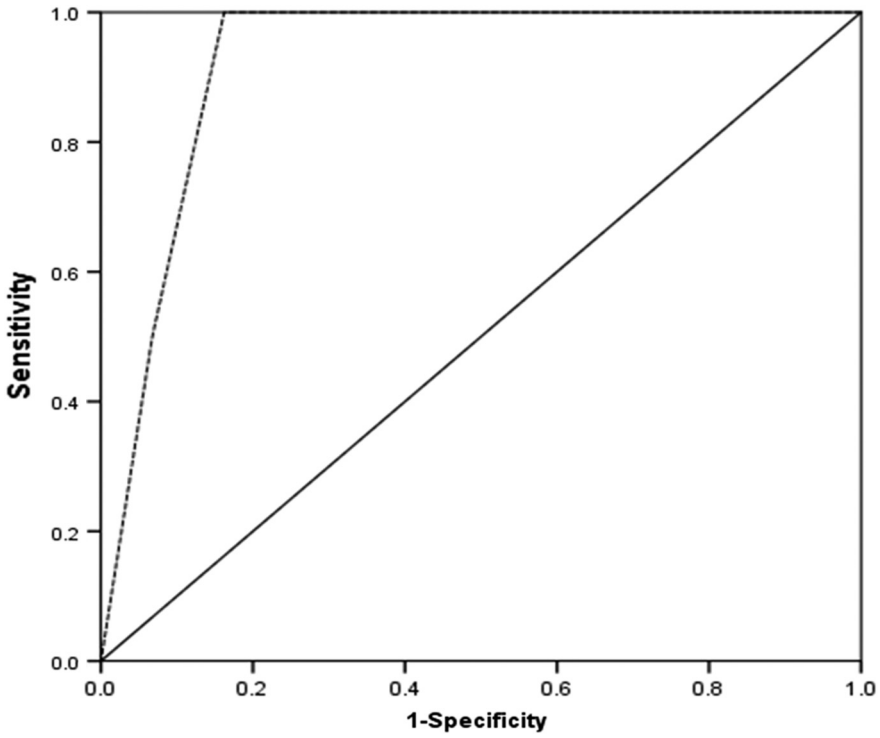

Figure 5. ROC curve analysis for TrkA immunostaining to predict *NTRK1* gene fusions using FISH. Cytoplasmic TrkA expression, yielding an area under the ROC curve of 0.926 (range: 0.864–0.987, 95% CI, $P = .001$) is shown.

Table 1. Relationship Between FISH and IHC for *NTRK* Fusion Gene Analysis in CRC Patients

Sum of Intensity and Proportion Score	[No.]	FISH for NTRK Fusion Gene		*P*
		Negative	Positive	
IHC cytoplasmic				
0–4	62	62 (100.0)	0 (0.0)	<.0001
5–6	18	12 (66.7)	6 (33.3)	
IHC membranous				
0–4	54	51 (94.4)	3 (5.6)	.384
5–6	26	23 (88.5)	3 (11.5)	

the results of IHC for TrkA and FISH and analyzed the ability of this screening method to detect *NTRK1* gene fusions in CRC samples, similar to the use of ALK IHC for detecting *ALK* fusion genes in non-small cell lung cancer.

In this study, we used an anti-TrkA monoclonal antibody that recognizes the C-terminal intracellular domain of TrkA as readout for *NTRK1* rearrangements in CRC. However, there have been no previous studies on using the intensity and proportion of cytoplasmic TrkA staining as a predictive marker for *NTRK1* gene fusions. Ardini et al. and Créancier et al. reported only strong cytoplasmic TrkA staining [7,8]. Lee et al. and Park et al. also reported only cytoplasmic TrkA staining [9,10]. Furthermore, the positive rate for cytoplasmic TrkA staining differed between the different studies (0.5–5.1%) [7–10]. Our data revealed a higher frequency of strong cytoplasmic TrkA staining rate (15%, 12/80) compared to the previous studies. We believe that these discrepancies may be partly attributed to the differences in the types of primary antibodies or specimens used. Two studies used a rabbit anti-TrkA monoclonal antibody (ab76291; Abcam, Cambridge, U.K.) [7,8]. Lee et al. used an anti-panTrk rabbit antibody (C17F1; Cell Signaling Technology). Park et al. and the present study used an anti-TrkA C-terminal monoclonal antibody (TA806413; OriGene, Rockville, MD, U.S.). Additionally, three of the studies [7,8,10] used tissue microarray technology, and one study used tissue blocks for performing TrkA IHC. As shown above, TrkA expression was observed to be heterogeneous. This may have been partly responsible for the high percentage of strong cytoplasmic TrkA expression observed in our study. Furthermore, for practical purposes, a false negative TrkA IHC results will be of concern due to the heterogeneous expression TrkA expression and *NTRK1* fusion genes. To overcome this issue, we needed to standardize the TrkA IHC method (primary antibodies, dilution and staining platform of immunohistochemistry) and size of specimen (size of tissue). Hechtman et al. reported that specific staining patterns for Trk expression were related with the subcellular localization of the fusion partners [13]. For example, in case of the *NTRK1-LMNA* fusion, immunostaining was observed in the nuclear membrane; however, in case of the *NTRK1-TPM* fusion, mixed cytoplasmic and membranous staining was observed [13]. In the present study, all *NTRK1* gene fusion-positive CRC samples displayed mixed cytoplasmic and membranous staining patterns for TrkA. Despite the limitations of our present study (small sample size procured from a single institution), we identified that strong cytoplasmic TrkA expression was associated with the presence of *NTRK1* gene fusions. Additionally, IHC for TrkA can be used as an effective screening method for detecting *NTRK1* gene fusions in CRC. Further multi-institutional studies encompassing larger cohorts, with other IHC methods and primary antibodies, are needed to confirm our present findings.

Table 2. Relationship Between *NTRK1* Fusion Gene Revealed by FISH Analysis and Clinicopathological Characteristics in 80 Patients With Colorectal Cancer

	[No.]	FISH for NTRK Fusion Gene		*P*
		Negative	Positive	
Age (years)	80	68.5 ± 1.23	59.5 ± 4.65	.051
Size (cm)	80	5.72 ± 0.24	5.40 ± 1.13	.720
Sex				
Male	44	41 (93.2)	3 (6.8)	1.000
Female	36	33 (91.7)	3 (8.3)	
Location				
Right colon	22	21 (95.5)	1 (4.5)	1.000
Left colon	58	53 (91.4)	5 (8.6)	
Histological type*				.582
Well	2	2 (100.0)	0 (0.0)	
Moderately	65	59 (90.8)	6 (9.2)	
Poorly	10	10 (100.0)	0 (0.0)	
Mucinous	3	3 (100.0)	0 (0.0)	
Invasion depth				.328
T3	56	56 (90.3)	6 (9.7)	
T4	18	18 (100.0)	0 (0.0)	
Perineural invasion				
Negative	40	37 (92.5)	3 (7.5)	1.000
Positive	40	37 (92.5)	3 (7.5)	
Lymphatic emboli				
Negative	50	44 (88.0)	6 (12.0)	.079
Positive	30	30 (100.0)	0 (0.0)	
Venous emboli				
Negative	70	64 (91.4)	6 (8.6)	1.000
Positive	10	10(100.0)	0 (0.0)	
Lymph node metastasis				.676
Negative	39	33 (94.9)	2 (5.1)	
Positive	41	36 (90.2)	4 (9.8)	
Microsatellite status				.480
MSS	70	65 (92.9)	5 (7.1)	
MSI-L	2	2 (100.0)	0 (0.0)	
MSI-H	8	7 (87.5)	1 (12.5)	

Acknowledgments

DYP and CHK conceived and designed this study. The experiments were performed by CHK, YC, and YJW. Histopathological slides analysis was carried out by DYP, SJL, AK, YK, and WYP. Clinical data was analyzed by HJJ and GAS. The manuscript was written by YC and edited by DYP and CHK. DYP supervised the research and obtained funding.

References

[1] Shin A, Kim KZ, Jung KW, Park S, Won YJ, Kim J, Kim DY, and Oh JH (2012). Increasing trend of colorectal cancer incidence in Korea, 1999-2009. *Cancer Res Treat* **44**, 219–226.

[2] Sobrero AF, Maurel J, Fehrenbacher L, Scheithauer W, Abubakr YA, Lutz MP, Vega-Villegas ME, Eng C, Steinhauer EU, and Prausova J, et al (2008). EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. *J Clin Oncol* **26**, 2311–2319.

[3] Cunningham D, Humblet Y, Siena S, Khayat D, Bleiberg H, Santoro A, Bets D, Mueser M, Harstrick A, and Verslype C, et al (2004). Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. *N Engl J Med* **351**, 337–345.

[4] Giantonio BJ, Catalano PJ, Meropol NJ, O'Dwyer PJ, Mitchell EP, Alberts SR, Schwartz MA, Benson III AB, and Eastern Cooperative Oncology Group Study E (2007). Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. *J Clin Oncol* **25**, 1539–1544.

[5] Cancer Genome Atlas N (2012). Comprehensive molecular characterization of human colon and rectal cancer. *Nature* **487**, 330–337.

[6] Seshagiri S, Stawiski EW, Durinck S, Modrusan Z, Storm EE, Conboy CB, Chaudhuri S, Guan Y, Janakiraman V, and Jaiswal BS, et al (2012). Recurrent R-spondin fusions in colon cancer. *Nature* **488**, 660–664.

[7] Ardini E, Bosotti R, Borgia AL, De Ponti C, Somaschini A, Cammarota R, Amboldi N, Raddrizzani L, Milani A, and Magnaghi P, et al (2014). The TPM3-NTRK1 rearrangement is a recurring event in colorectal carcinoma and is associated with tumor sensitivity to TRKA kinase inhibition. *Mol Oncol* **8**, 1495–1507.

[8] Creancier L, Vandenberghe I, Gomes B, Dejean C, Blanchet JC, Meilleroux J, Guimbaud R, Selves J, and Kruczynski A (2015). Chromosomal rearrangements involving the NTRK1 gene in colorectal carcinoma. *Cancer Lett* **365**, 107–111.

[9] Lee SJ, Li GG, Kim ST, Hong ME, Jang J, Yoon N, Ahn SM, Murphy D, Christiansen J, and Wei G, et al (2015). NTRK1 rearrangement in colorectal cancer patients: evidence for actionable target using patient-derived tumor cell line. *Oncotarget* **6**, 39028–39035.

[10] Park DY, Choi C, Shin E, Lee JH, Kwon CH, Jo HJ, Kim HR, Kim HS, Oh N, and Lee JS, et al (2016). NTRK1 fusions for the therapeutic intervention of Korean patients with colon cancer. *Oncotarget* **7**, 8399–8412.

[11] Chao MV (2003). Neurotrophins and their receptors: a convergence point for many signalling pathways. *Nat Rev Neurosci* **4**, 299–309.

[12] Kaplan DR and Miller FD (2000). Neurotrophin signal transduction in the nervous system. *Curr Opin Neurobiol* **10**, 381–391.

[13] Hechtman JF, Benayed R, Hyman DM, Drilon A, Zehir A, Frosina D, Arcila ME, Dogan S, Klimstra DS, and Ladanyi M, et al (2017). Pan-Trk immunohistochemistry is an efficient and reliable screen for the detection of NTRK fusions. *Am J Surg Pathol*. https://doi.org/10.1097/PAS.0000000000000911.

[14] Ou SH (2011). Crizotinib: a novel and first-in-class multitargeted tyrosine kinase inhibitor for the treatment of anaplastic lymphoma kinase rearranged non-small cell lung cancer and beyond. *Drug Des Devel Ther* **5**, 471–485.

[15] Conklin CM, Craddock KJ, Have C, Laskin J, Couture C, and Ionescu DN (2013). Immunohistochemistry is a reliable screening tool for identification of ALK rearrangement in non-small-cell lung carcinoma and is antibody dependent. *J Thorac Oncol* **8**, 45–51.