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Original contribution

Tropomyosin receptor kinases B and C are tumor progressive and metastatic marker in colorectal carcinoma☆

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Summary Members of the tropomyosin receptor kinase (Trk) family have a high affinity for neurotrophins and regulate neuronal survival. The role of Trks in cancer is still controversial. The expression and role of TrkB and TrkC were examined in colorectal cancer (CRC). Immunohistochemical analysis of TrkB and TrkC was performed in 133 patients with CRC. Using human CRC cell lines, expression of vascular endothelial growth factor (VEGF) and transforming growth factor β, cell growth, invasion, and apoptosis were examined by knockdown methods. Immunohistochemistry showed positive results of TrkB and TrkC (23.3% and 12.8%, respectively). TrkB expression was associated with local progression ($P = .0284$), clinical stage ($P = .0026$), nodal metastasis ($P = .0068$), and peritoneal metastasis ($P = .0026$). TrkC expression was only related to liver metastasis ($P = .0001$). Coexpression of TrkB or TrkC and their ligands was found in 80.6% and 82.4% of cases, respectively. In vitro analysis using human CRC cells showed that TrkB positively regulated gene expression of VEGF-A ($P < .05$) and VEGF-C ($P < .05$), whereas TrkC suppressed transforming growth factor β expression ($P < .05$). TrkB and TrkC induced cell growth ($P < .05$) and invasion ($P < .05$), respectively. Both TrkB and TrkC showed antiapoptotic effect ($P < .05$). These results suggest that TrkB and TrkC have a tumor progressive function and may be a useful diagnostic and therapeutic target in CRC.

Abbreviations: CRC, colorectal cancer; Trk, tyrosine kinase receptor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; NT, neurotrophin; BMP, bone morphogenetic protein; VEGF, vascular endothelial growth factor; TGF, transforming growth factor; PI3K, phosphatidylinositol 3′-kinase; MAPK, mitogen-activated protein kinase; mTOR, mammalian target of rapamycin; MMP, matrix metalloproteinase; EMT, epithelial-mesenchymal transition.

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1. Introduction

Colorectal cancer (CRC) is the third most common malignancy worldwide [1] and is responsible for more than 500 000 deaths annually [2]. The overall 5-year survival rate decreases from approximately 80% in patients without nodal metastases to 50% in patients with nodal metastasis [3]; it is less than 10% in those with distant metastasis [4]. Liver metastasis is found in 30% to 60% of metastatic cases, and most patients who have liver metastases die within 5 years of diagnosis [5]. Therefore, early detection of CRC is important, and elucidation of the detailed molecular mechanism promoting metastasis of CRC is critical.

Ligands for the tropomyosin receptor kinase (Trk) family are neurotrophins (NTs); TrkA binds to nerve growth factor, TrkB binds to brain-derived neurotrophic factor (BDNF) and NT-4/5, and TrkC binds to NT-3 [6,7]. NTs initiate autophosphorylation at the extracellular domain of Trks by binding to Trks and promoting downstream signaling transduction pathways [7]. The Trk family has been reported as regulating neuronal survival and differentiation [8]. Trks also act as oncogenes; TrkA overexpression has been observed in thyroid carcinoma [9,10]. Higher expression levels of TrkB have been found in many tumors and are associated with more aggressive tumor behavior [11,12]. In CRC, TrkC directly binds to the bone morphogenetic protein type II receptor and inhibits bone morphogenetic protein signaling [13]. Moreover, TrkB and TrkC inhibit apoptosis in ovarian cancer and neuroblastoma cells via phosphatidylinositol 3′-kinase (PI3K)–AKT signaling, respectively [14,15]. However, it has been reported that TrkB expression is down-regulated in prostate cancer [16]. Furthermore, TrkC plays an favorable role in medulloblastoma, leading to a good clinical outcome [17], and cases of neuroblastoma with high expression of TrkA or TrkC have a better prognosis [18,19]; thus, the role of Trks in tumors is still controversial.

It has been reported recently that TrkB induces angiogenesis by activation of vascular endothelial growth factor (VEGF)-A in neuroblastoma [20] and that TrkC suppresses transforming growth factor (TGF) β signaling in breast cancer [21]. We also confirmed that TrkB and TrkC promote tumor progression, nodal metastasis, and induction of angiogenesis and lymphangiogenesis in oral cancer [22]; however, not much information is available about the expression pattern and clinicopathologic significance of Trks in CRC. In this study, we examined the expression of TrkB/C and the effects on the VEGF family and TGF-β signaling by using clinical samples and cell lines of CRC.

2. Materials and methods

2.1. Tissue samples

Formalin-fixed, paraffin-embedded 133 cases of primary CRCs (92 men and 41 woman; age range, 48-79 years; means, 68.7 years), and 9 fresh-frozen specimens each of CRC and noncancerous colorectal mucosa were randomly selected from Nara Medical University Hospital, Kashihara, Japan, and Miyoshi General Hospital, Miyoshi, Japan. All cases received no preoperative treatment. Tumor staging and the histology of CRCs were classified according to TNM classification and World Health Organization classification, respectively. Medical records and prognostic follow-up data were obtained from the patient database managed by the hospital. Because written informed consent was not obtained, identifying information for all samples was removed before analysis for strict privacy protection; the procedure was in accordance with the Ethical Guidelines for Human Genome/Gene Research enacted by the Japanese Government.

2.2. Immunohistochemistry

Consecutive 3-μm sections were cut from each block, and immunohistochemical analysis was performed as we described previously [23,24]. An immunoperoxidase technique was done after antigen retrieval with microwave treatment (95°C) in citrate buffer (pH 6.0) for 45 minutes or pepsin (DAKO, Carpinteria, CA) treatment for 20 minutes. After endogenous peroxidase block by 3% H_2O_2-methanol for 15 minutes, specimens were rinsed with phosphate-buffered saline (PBS) 3 times. Anti-TrkB anti-TrkC antibody (Santa Cruz Biotechnology, Santa Cruz, CA), anti-BDNF antibody (R&D Systems, Temecula, CA), and anti–NT-3 antibody (CHEMICON, Minneapollis, MN), diluted by 0.5 μg/mL were used for primary antibody. After a 12-hour incubation at room temperature, specimens were rinsed with PBS 3 times and treated for an hour at room temperature with the secondary antibody peroxidase-conjugated antimouse or antirabbit antibody (MBL, Nagoya, Japan) diluted at 0.5%. The specimens were then rinsed with PBS 3 times and color developed with diaminobenzidine (DAB) solution (DAKO). After washing, specimens were counterstained with Meyer hematoxylin (Sigma Chemical, St Louis, MO). Immunostaining of all samples was performed at the same conditions of antibody reaction and DAB exposure. For evaluation of Trks expression, we observed 20 microscopic fields at 100-fold magnification. Immunoreactivity of Trks was classified according to Allred score (AS) [25]. We divided the immunoreactivity into 4 grades by AS; grade 0, AS is 0; grade 1, AS is 2 to 4; grade 2, AS is 5 to 6; and grade 3, AS is 7 to 8. Cases with grade 2 to 3 staining were defined as TrkB or TrkC positive [23,24,26].

2.3. Cell culture

Human CRC cell lines WiDr, Colo320, HT29, and LoVo cells were obtained from Health Science Research Resources Bank and maintained in Dulbbeco modified Eagle medium (Wako Pure Chemical, Osaka, Japan) supplemented with 10% fetal bovine serum (Sigma Chemical) under the conditions of 5% CO_2 in air at 37°C.