

RESEARCH HIGHLIGHT

Wnt signaling in idiopathic carpal tunnel syndrome

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Carpal tunnel syndrome (CTS) is the most frequently reported entrapment neuropathy; however, the exact pathological mechanism of CTS remains unknown. In a recent paper published in the *Journal of Orthopaedic Research*, we investigated the associations between Wnt signaling and the etiology of idiopathic CTS (ICTS). We compared the expression levels of genes encoding Wnt1, 2, 3, 4, 5a, 5b, 6, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11, and 16 in the flexor tenosynovium between ICTS patients and controls, and we also evaluated whether an association exists between Wnt signaling and cell proliferation factors, such as estrogen-responsive finger protein, epidermal growth factor receptor, heparin binding-epidermal growth factor-like growth factor, insulin-like growth factor-1, and vascular endothelial growth factor (VEGF). To compare the cell proliferation potency, expression levels of MIB-1 protein were also measured. We found that Wnt9a gene expression in the flexor tenosynovium is more prominent in ICTS patients. A positive correlation was observed in only ICTS patient group for the gene expression of Wnt9a and VEGF in the flexor tenosynovium. There were no relationships between the expression levels of Wnt9a and fibroblast proliferation in either group. These results indicate that Wnt9a may be involved in the expression of VEGF in ICTS.

Keywords: Idiopathic carpal tunnel syndrome; Wnt signal; Vascular endothelial growth factor

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Carpal tunnel syndrome

Carpal tunnel syndrome (CTS) is the most common type of entrapment neuropathy. The prevalence rate of undetected CTS was reported to be 5.8% in adult women [1], and majority of the reported CTS cases were idiopathic [2]. Certain risk factors have been associated with idiopathic CTS (ICTS) [3]. Some cases on the involvement of flexor tenosynovium fibrosis with ICTS have been reported. Chikenji et al. (2014) reported that fibrotic factors, such as transforming growth factor (TGF)- β 1, type III collagen, and expression levels of connective tissue growth factor (CTGF),

were increased in CTS patients [4]. Ettema et al. (2004) also reported that the expression levels of type III collagen were increased in CTS patients [5]. On the other hand, some studies have reported vascular abnormalities in the flexor tenosynovium. Lluch et al. (1992) detected the presence of blood vessel wall destruction and angiogenesis accompanied by a degree of edema in CTS patients [6]. Hirata et al. (2004) suggested that angiogenesis occurs as a regenerative attempt during the intermediate CTS phase in response to vascular insufficiencies due to severe vascular narrowing [7]. Minimum inflammatory changes have been observed in the

flexor tenosynovium, which is a characteristic of ICTS [8]. However, the exact pathological mechanism of ICTS remains unknown.

Wnt signaling

Wnt signaling controls initial cell development, morphogenesis, and organogenesis as well as postnatal cell proliferation, differentiation, and activity, and 19 types of Wnt ligands have been identified in humans and mice to date [9]. Wnt signaling pathways include the canonical Wnt signaling pathway, non-canonical cell polarity pathway, and non-canonical Wnt-calcium pathway to control cell proliferation, differentiation, activity, and polarity [10]. Therefore, Wnt signaling pathway abnormalities can potentially cause various diseases [11]. Beuge *et al.* (2015) reported that the expression of Wnt5a was upregulated in Dupuytren disease patients [12]. Wei *et al.* (2012) demonstrated that Wnt3a stimulates the expression of TGF- β 1 in systemic sclerosis (SSc) patients [13]. However, there have been no reports on the relationship between Wnt signaling and ICTS. Therefore, we investigated this relationship in our study [14].

Relationship between CTS and Wnt signaling

We evaluated nine ICTS patients (average age, 77 years) and nine controls with distal radius fractures without any ICTS symptoms (average age, 77 years). The patients included in this study were only postmenopausal women. We evaluated tenosynovial tissue biopsy samples collected from inside the carpal tunnel during open carpal tunnel release for ICTS or during open reduction and internal fixation for distal radius fracture. Total RNA from tenosynovial tissues was extracted using the RNeasy Plus Mini kit (Qiagen N.V., Venlo, Netherlands) following the manufacturer's protocol. Concentration and purity of the total RNA samples were measured using the NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE). Total RNA was transcribed into complementary DNA (cDNA) using the High Capacity RNA-to-cDNA Master Mix (Applied Biosystems, Foster City, CA) following the manufacturer's protocol. The cDNAs were used for quantitative real-time polymerase chain reaction (PCR). We compared the expression levels of genes encoding Wnt1, 2, 3, 4, 5a, 5b, 6, 7a, 7b, 8a, 8b, 9a, 9b, 10a, 10b, 11, and 16 between the two groups using quantitative real-time PCR.

The result was that the gene expression level of Wnt9a was significantly higher in the tenosynovial tissue of ICTS patients than in that of the controls. For Wnt9a, immunohistochemical staining was performed with a primary anti-Wnt9a antibody (1:100 dilution, ab125957; Abcam,

Cambridge, UK) to compare the protein expression levels in the groups. Antibodies were visualized using diaminobenzidine tetrahydrochloride (ChemMate DAB+Chromogen, DakoCytomation). Four randomly chosen fields were analyzed at 20 \times light microscope magnification, and were counted the numbers of positive cells. The expression level of Wnt9a was quantified with ImageJ 1.46r software package (<http://imagej.nih.gov/ij>) at a threshold of 120 px in each image. Immunohistochemical analysis also showed that Wnt9a positive cells were significantly higher in the tenosynovial tissue of ICTS patients than in those of the controls. In particular, the expression of Wnt9a around the blood vessels was a characteristic finding in ICTS patients but not in controls.

Little is known about the effect of Wnt9a. Wnt9a is best known to have an important role in joint formation [15, 16]. Spater *et al.* (2006) also reported that the canonical Wnt/ β -catenin signaling pathway is required for joint integrity with Wnt9a having an important role [17].

Gingery *et al.* (2014) reported that fibrotic factors, such as the expression of type I and type III collagen in the flexor tenosynovium, are increased in CTS patients [18]. Several studies have reported that canonical Wnt/ β -catenin signaling pathway affects fibrosis. Akhmetshina *et al.* (2012) reported that fibrosis could not be prevented completely in SSc patients, blocking the Smad signaling downstream of TGF- β , but it was possible to prevent fibrosis by adding DKK1 that antagonizes the canonical Wnt/ β -catenin signaling pathway [19]. Other studies have reported that TGF- β /Smad signaling pathway and canonical Wnt/ β -catenin signaling pathways can cross-talk with each other and affect the fibrosis [20]. It is interesting to note whether Wnt9a is associated with flexor tenosynovium fibrosis in ICTS. However, further study is needed to clarify this.

Relationship between CTS and cell proliferation factors

We also investigated the expression of genes encoding cell proliferation factors, such as estrogen-responsive finger protein (Efp), epidermal growth factor receptor (EGFR), heparin binding-epidermal growth factor-like growth factor (HB-EGF), insulin-like growth factor-1 (IGF-1), and vascular endothelial growth factor (VEGF), using quantitative real-time PCR. In addition, we compared the expression levels of MIB-1 protein to evaluate the cell proliferation potency between the two groups using immunohistochemical staining. Immunohistochemical staining for MIB-1 was performed using an anti-human MIB-1 antigen (1:100 dilution, M7240; Dako Co., Glostrup, Denmark) and the I-VIEW DAB universal kit (Roche, Rotkreuz, Switzerland) following the manufacturer's

protocol. Five randomly chosen fields were analyzed at 20× light microscope magnification, and were measured the percentage of positive cells. The expression level of MIB-1 was quantified with Ariol SL200 (Leica Biosystems, Wetzlar, Germany).

No significant differences were observed in the gene expression of the cell proliferation factor between the ICTS patient and control groups. On the other hand, a positive correlation was observed between the expression of Wnt9a and VEGF genes in the flexor tenosynovium of ICTS patients ($R = 0.69$, $p < 0.05$). The mean MIB-1-positive area showed no difference between ICTS patients and controls ($p = 0.79$). There also was no correlation between the number of Wnt9a-positive cells and the percentage of MIB-1-positive cells in the two groups ($p = 0.83$).

Regarding the expression of VEGF in ICTS, Hirata *et al.* (2004) reported that VEGF directly promotes angiogenesis, vessel wall thickening, and intimal hyperplasia in the intermediate phase of CTS [7]. Donate *et al.* (2009) reported that neoangiogenesis consists of anomalous vessels and may be triggered from various cell types secreting VEGF [21]. Regarding the relationship between the expression of Wnt9a and VEGF, Wnt9a has been reported to be involved in the expression of VEGF-flk1 on hepatocyte differentiation in chicken embryos [22]. Our study showed that the expression of Wnt9a around the blood vessels was a characteristic finding in ICTS patients, and, therefore, there is a possibility that the expression of Wnt9a affects the expression of VEGF in ICTS patients. On the other hand, we could find no relationship between the expression of Wnt9a and fibroblast proliferation in ICTS patients.

Conclusions

Gene expression of Wnt9a in the flexor tenosynovium was more prominent in ICTS patient group than in the control group. There were no differences between the ICTS patient and control groups regarding the expression of cell proliferation factors, such as Efp, EGFR, HB-EGF, IGF-1, and VEGF, or the expression of MIB-1 protein. No relationships were observed between the mRNA expression of Wnt9A and cell proliferation factors other than VEGF or between the protein expression levels of Wnt9A and MIB-1. A positive correlation was observed only in the ICTS patient group was for the expression of Wnt9a and VEGF genes in the flexor tenosynovium.

Conflicting interests

The authors have declared that no competing interests exist.

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