We demonstrate a novel interaction of the nephroblastoma overexpressed gene (NOV), a member of the CCN gene family, with the Notch signaling pathway. NOV associates with the epidermal growth factor–like repeats of Notch1 by the CT (C-terminal cysteine knot) domain. The promoters of HES1 and HES5, which are the downstream transducers of Notch signaling, were activated by NOV. Expressions of NOV and Notch1 were concomitant in the presomitic mesoderm and later in the myocytes and chondrocytes, suggesting their synergistic effects in mesenchymal cell differentiation. In C2/4 myogenic cells, elevated expression of NOV led to down-regulation of MyoD and myogenin, resulting in inhibition of myotube formation. These results indicate that NOV-Notch1 association exerts a positive effect on Notch signaling and consequently suppresses myogenesis.

Nephroblastoma overexpressed gene (NOV) was first identified as an overexpressing gene in virus-induced avian nephroblastoma (1), and its orthologues were later isolated from Xenopus, rat, mouse, and human (1–6). NOV belongs to the CCN gene family, which is constituted by connective tissue growth factor (CTGF), cysteine-rich 61 (Cyr61), NOV, and other related genes (7–11). NOV encodes a putative secretory protein with 343–357 amino acids that contains four conserved modular domains with sequence similarities to insulin-like growth factor–binding protein, von Willebrand type C, and C-terminal cysteine knot (CT) domain (12). CCN genes have not been found in invertebrates as yet, and each domain is encoded by a separate exon, implying that the CCN genes might arise from exon shuffling in the course of vertebrate evolution. These modular structural domains are shared among the CCN family members, except for WISP-2/COP-1, which lacks the CT module (13), and WISP1v, which lacks the von Willebrand type C module (14). The CCN gene family and a number of related genes now appear to constitute an emerging multigene superfamily.

Some CCN proteins have been demonstrated to possess a growth factor–like activity and regulate cell growth and tissue formation. For example, CTGF is a matrix-associated, heparin-binding protein that mediates cell proliferation, migration, and adhesion. CTGF also functions as a downstream mediator of TGF-β and stimulates proliferation and extracellular matrix synthesis of fibroblasts (15, 16). Compared with CTGF, the biological activity of NOV remains poorly understood. Overexpression of NOV inhibits the proliferation of chicken embryonic fibroblasts, suggesting its negative properties on cell growth. On the contrary, human NOV protein stimulates the proliferation of fibroblasts and also induces protein tyrosine phosphorylation, implying its positive effect on cell proliferation (5). N-terminal truncated form of NOV can transform chicken embryonic fibroblasts by its oncogenic activity, and aberrant expression of NOV is associated with the development of several tumors of different origins including Wilms’ tumor, renal cell carcinoma, neuroblastoma, glioblastoma, adrenocortical carcinoma, and musculoskeletal tumors (17–20). During normal development, NOV is expressed in a wide variety of tissues. The major sites of NOV expression include the notochord, central nervous system, kidney, adrenal cortex, muscle, and cartilage (21–28). Although a growing body of evidence suggests that NOV plays an important role in the development of various tissues, the mechanisms of its function remain unclear. A search has been undertaken for molecules that interact with NOV. Two proteins were confirmed to physically associate with NOV. One is fibulin-1C, an extracellular matrix protein that mediates cell adhesion, which suggests the involvement of NOV in cell adhesion signaling (29). The other is rpβ7, a subunit of RNA polymerase II, implying that NOV might regulate transcription in nucleus (30). More recently, NOV was shown to co-localize with connexin 43, suggesting that they might also
interact and influence the formation of gap junction (18). Although the biological activities of NOV are attributed to interactions with these molecules to some extent, two-hybrid system analysis has indicated that other factors may interact with NOV and would likely regulate its downstream events.

We recently demonstrated that NOV is expressed in Hensen's node, notochord, and the floor plate in early chick embryogenesis (31). The expression of NOV is also detected in the dermomyotome (28). Interestingly, the expression in the dermomyotome coincides with that of transmembrane receptor, Notch and its ligand, Delta. The Notch signaling pathway plays a crucial role in cell proliferation, differentiation and fate determination in various tissues. The expression of Notch frequently overlaps with NOV expression. For example, both genes are expressed in chondrocytes and are supposed to participate in cartilage formation (26, 32). NOV is also involved in vascular formation (33), and its role in response to injury has been postulated, in which prominent up-regulation of NOV was observed in smooth muscle cells after balloon catheter injury of rat carotid artery (34). In this type of experimental model, remarkable up-regulation of NOV was observed after balloon catheter injury of rat carotid artery (34). In this type of experimental model, remarkable up-regulation of NOV was observed after balloon catheter injury of rat carotid artery (34). In this type of experimental model, remarkable up-regulation of NOV was observed after balloon catheter injury of rat carotid artery (34).

**DELETION STUDIES**

The C-terminal CT domain of NOV is required for the interaction with Notch1. Deletion constructs of NOV were created, and their ability to associate with Notch1 were examined. A, schematic illustration of the NOV deletion constructs. C-terminal HA-tagged Notch constructs were created from wild type Notch1 (Noutwt). The signal sequence of IgG was added to the N-terminal deletion constructs (Nov-d1, Nov-d2, Nov-d4). The numbers denote the positions of the amino acids. IGFBP, insulin-like growth factor-binding protein-like domain; VWC, von Willebrand type C factor-like domain; TSP1, thrombospondin type 1-like domain; CT, C-terminal cysteine knot domain.

**Cell Culture and In Vitro Transfection**

Cells were transfected with total 1 μg of DNA in 12- or 24-well culture vessels using LipofectAMINE 2000 (Invitrogen) per the manufacturer's instruction. C2/4-NOV and C2/4-Mock were established by Geneticin (Invitrogen) selection.

**Baculovirus Expression System**

The HA-tagged NOV fragment was ligated into pFastBac1 (Invitrogen). Bacmid production and transfection to High Five cells were performed per the manufacturer's instruction. NOV protein secreted in the culture medium was concentrated using Microcon YM10 (Millipore).

**EXPERIMENTAL PROCEDURES**

**Genes**—Murine Notch1 was a gift from J. Nye (Northwestern University, Chicago). NICD (Notch-d4) was a gift from E. Robey (University of California, Berkeley). Delta1, Delta-d1, and Serrate1 were previously described (36). The C terminus of NOV was tagged with a Flag or an HA epitope by ligating the EcoRI-StuI fragment of the original NOV clone into pCMV-Tag4 (Stratagene) or pTagHA (personal product), respectively. The restriction digestion by StuI removes the C-terminal sequence encoding 12 amino acids that are out of the CT domain and show poor homologies among species and the CCN family genes (see Fig. 2A). NOV-d1, NOV-d2, NOV-d3, and NOV-d4 fragments were amplified from the HA-tagged NOV by PCR and ligated to pTagHA or pSecTag2 (Invitrogen) in such a manner as to have a signal sequence at their N-terminal ends and the HA epitope at their C-terminal ends. The full-length Notch1 was the wild type tagged with a Flag epitope was described previously (36). Notch-d1 was made by removing the ScuI fragment from the full-length Notch1. Notch-d2 was made by ligating the EcoRI-ScuI fragment of Notch1 into the EcoRI-EcoRV site of pCMV-Tag4. Notch-d3 was made by ligating the ScuI fragment of Notch1 into the Smal site of pFLAG-CMV-1 (Sigma). Notch-d4 is the same with that previously described as NICD (36). MyoD was a gift from A. Koseki (Chiba University, Chiba, Japan).

**Cell Culture and In Vitro Transfection**—C2/4a was provided by S. Matsuda (Kyoto University, Kyoto, Japan). HEK293 or C2/4 cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (Sigma) and antibiotics. For induction of differentiation, the culture medium was changed to Dulbecco's modified Eagle's medium containing 2% horse serum (Sigma, lot 20K8408). Transfection was performed with total 1 μg of DNA in 12- or 24-well culture vessels using LipofectAMINE 2000 (Invitrogen) per the manufacturer's instruction. C2/4-NOV and C2/4-Mock were established by Geneticin (Invitrogen) selection.

**Baculovirus Expression System**—The HA-tagged NOV fragment was ligated into pFastBac1 (Invitrogen). Bacmid production and transfection to High Five cells were performed per the manufacturer's instruction. NOV protein secreted in the culture medium was concentrated using Microcon YM10 (Millipore).
NOV Associates with Notch

**RESULTS**

**NOV Associates with Notch1**—To examine the possibility that NOV participates in the Notch signaling pathway, we investigated whether NOV associates with Notch1 or its ligands, Delta1 and Serrate1, using an immunoprecipitation assay. The C terminus of NOV was tagged with an HA epitope, and the C terminus of Notch1, Delta1, and Serrate1 were each tagged with a Flag epitope. Immunoprecipitation of Notch1 from the protein extracts of HEK293 cells co-transfected with NOV and Notch1 recovered a complex containing NOV (Fig. 1A). NOV protein was not recovered in the absence of Notch1 protein (Fig. 1A). The levels of NOV in the lysates were comparable. These results indicate that NOV associates with Notch1. We also performed immunoprecipitation of NOV from protein extracts of the cells co-transfected with Flag-tagged NOV and HA-tagged Notch1. Full-length Notch1 protein was recovered from the immunoprecipitant of NOV, which confirmed the NOV-Notch1 association (Fig. 1B). The small fragment that represents a cleaved Notch1 product with the transmembrane and intracellular domain (Notch tm+ic) was not co-immunoprecipitated with NOV, which implies that NOV associates with the extracellular domain of Notch1 (Fig. 1B). The associations of NOV-Delta1 or NOV-Serrate1 were also detected but at less than 50-fold the level of NOV-Notch1 (Fig. 1A).

**The CT Domain of NOV Is Required for Association with Notch1**—Like other CCN family members, NOV consists of four modules, each of which appears to be involved in interaction with different molecules. To determine which module is engaged in the association with Notch1, we made a series of deletion constructs of NOV and examined their association with Notch1 (Fig. 2A). Because the wild type NOV has a secretory character, the signal sequence of IgG was appended to the N-terminal deletion constructs (NOV-d1, NOV-d2, and NOV-d4). Immunoprecipitation analysis revealed that NOV-d1, which lacks the first module, and NOV-d2, which lacks the first

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**Immunoprecipitation and Western Blot Analysis**—Cells were passively lysed in TNTC buffer (100 mM Tris-Cl, pH 7.6, 150 mM NaCl, 1% Triton X-100, 1 mM CaCl2, and Complete protease inhibitor (Roche Molecular Biochemicals)) 24 h after transfection. In the experiments to test calcium dependence, TNT buffer (100 mM Tris-Cl, pH 7.6, 150 mM NaCl, 1% Triton X-100) containing EGTA was also used. Cell lysate was pre-absorbed to CL4B-agarose beads and incubated with anti-Flag M2 affinity gel (Sigma) at 4 °C for 1 h. The beads were washed three times with lysis buffer, 8 or 10% SDS-PAGE was conducted with SDS gel loading buffer containing 100 mM dithiothreitol. The proteins were transferred to Hybond ECL nitrocellulose membrane (Amersham Pharmacia Biotech). Immunodetection was performed as described previously (36). The antibodies used were peroxidase-conjugated anti-HA (clone 3F10, Roche Molecular Biochemicals), peroxidase-conjugated anti-M2 (M2, Sigma), anti-phosphotyrosine (clone PT-66, Sigma), anti-human smooth muscle actin (clone 1A4, Dako), anti-porcine desmin (clone DE-R-11, Dako), and anti-rat myogenin (clone F5D, Developmental Studies Hybridoma Bank (DSHB)). Densitometric analysis was conducted on the scanned film image using Photoshop 6.0 (Adobe). In brief, the signal intensities were calculated by integrating the blackness (0–255) of each pixel of the band images. The values of NOV co-precipitated with Notch were divided by the values of Notch in the cell lysates. Note that the values are merely relative indices without any physical meaning.

**Northern Blot Analysis**—RNA was extracted from the C2/4 cells cultured in the differentiation-inducing medium using Trizol (Invitrogen). Ten micrograms of total RNA were loaded to each lane. Standard Northern blot procedures were adopted and hybridization was performed using [32P]-dCTP-labeled NOV, Notch-Delta1, or MyoD cDNA fragment. Detection and imaging of the radioactive signals were conducted using the image scanner BAS2500 (Fuji film).

**Luciferase Activity Assay**—The reporter plasmids of HES1 and HES5 promoters, HES1-luc and HES5-luc, were gifts from R. Kageyama (Kyoto University, Japan). Luciferase activity was measured using the dual-luciferase reporter assay system (Promega) and the Luminosensor AB-2200 (Atto) per the manufacturers’ instructions. All experiments were performed in triplicate, and firefly luciferase activity was normalized by co-transfected Renilla luciferase activity (pRL-CMV, a gift from Y. Mochida, Tokyo Medical and Dental University, Japan).

**In Situ Hybridization**—In situ hybridization using digoxigenin-labeled RNA probe was performed as described previously (31, 37).

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**Fig. 3. NOV associates with the EGF repeats of Notch1.** Deletion constructs of Notch1 were created, and their abilities to associate with NOV were examined. A, schematic illustration of the Notch1 deletion constructs. Notch-d1 lacks the 10th–36th EGF motifs. Notch-d2 consists of the N-terminal region that includes the 1st–9th EGF motifs. Notch-d3 consists of the 10th–36th EGF motifs with a signal sequence. Notch-d4 consists of the intracellular domain and has a constitutive activity of the Notch signal transduction. The locations of the Flag tag are shown as filled boxes in the illustration. EGF, epidermal growth factor-like repeats; Lin, Lin/Notch repeats; TM, transmembrane domain; Ank, CDC10/ankyrin repeats. B, HEK293 cells were co-transfected with the Flag-tagged Notch1 and the HA-tagged NOV constructs. Immunoprecipitation was performed with anti-Flag M2 antibody (IP:Flag). NOV was co-precipitated with Notch, Notch-d1, Notch-d2, and Notch-d3 but not with Notch-d4. The numbers at the bottom of each lane are the results of densitometric analysis and represent the relative amount of NOV captured by each Notch protein. WB, Western blot. C, NOV-Notch1 association is Ca2+-independent. The cell lysate from HEK293 cells co-transfected with the HA-tagged NOV and the Flag-tagged Notch1 were immunoprecipitated by anti-Flag M2 antibody in the TNT buffer. The immunoprecipitant was divided into five aliquots, which were incubated for 30 min at room temperature with 40 μl of the TNT buffer, the TNT buffer containing 1–2 mM CaCl2, or EGTA. The protein associated with the beads and the protein in the supernatants (sup) were blotted separately and immunodetected with anti-HA antibody. The NOV-Notch1 complexes were stable in the buffer without Ca2+ and did not dissociate even after EGTA treatment.
lacks the 10th–36th EGF motifs (Fig. 3B). NOV associated with Notch-d2, which comprises only the first to ninth EGF motifs, and also with Notch-d3, which comprises only the 10th–36th EGF motifs (Fig. 3B). The intracellular domain of Notch1 (Notch-d4) did not associate with NOV (Fig. 3B). Densitometric analysis was performed to evaluate the amount of NOV captured by each Notch product. As for Notch-d1, -d2, and -d3, the amounts of the co-immunoprecipitated NOV were proportional to the amounts of each Notch deletion product. On the other hand, the full-length Notch1 captured NOV 10-fold more efficiently than those artificially engineered Notch deletion products (Fig. 3B, bottom). These results indicate that NOV tends to associate preferentially with the EGF repeat region of wild type Notch1.

**NOV-Notch1 Association Is Ca2+-independent**—Subsets of EGF domains contain calcium ion-dependent EGF motifs (38–40). Notch has 36 EGF motifs, of which 21 are potentially Ca2+-binding. We examined whether the loss of Ca2+ alters the affinity of NOV-Notch1 association. HEK293 cells co-transfected with NOV and Notch1 were lysed with TNT lysis buffer, and immunoprecipitation of Notch1 was performed. The immunoprecipitant was divided into five aliquots, which were incubated for 30 min in the TNT buffer or in TNT buffer containing 1 mM or 2 mM Ca2+ or 1 mM or 2 mM EGTA, respectively. NOV protein that was kept captured on the beads with Notch or was released in the supernatants was analyzed by immunoblot.

NOV-Notch1 complex was maintained in the buffer without Ca2+, and EGTA treatment did not dissociate the NOV-Notch1 complex, suggesting that NOV-Notch1 association is independent of Ca2+ (Fig. 3C).

**NOV Enhances HES1 and HES5 Promoter Activation via**

and the second modules, associated with Notch1, whereas NOV-d3 and NOV-d4, both of which lack the fourth module, did not associate with Notch1 (Fig. 2B). These results indicate that the CT domain is required for association with Notch1.

**NOV Binds to the EGF Motifs of Notch1**—To determine the region of Notch1 involved in association with NOV, we created deletion constructs of Notch1 and examined the association with NOV (Fig. 3A). NOV associated with Notch-d1, which

**Fig. 4. NOV enhances HES1 and HES5 promoter activities through interaction with Notch1.** A, C2/4 cells were transfected with 0.1 μg of HES1-luc or HES5-luc, 0.005 μg of pRL-EF, and the indicated amounts of NOV. The total amount of plasmid was equalized with the empty mock vector. Twenty-four hours later, the cells were lysed and assayed for luciferase activity. HES1 and HES5 promoter activities were increased by NOV in a dose-dependent manner. RLU, relative luciferase unit. B, C2/4 cells were cotransfected with 0.1 μg of HES1-luc or HES5-luc, 0.005 μg of pRL-EF, and 0.2 μg of the indicated plasmids, and a luciferase activity assay was performed. Transfection with NOV or NOV-d1 resulted in HES1 and HES5 activation (second and third bars from left), whereas NOV-d3 did not show this effect (fourth bars). Cotransfection with the dominant negative form of Delta1 (Notch-d1) cancelled the effect of NOV (fifth bars). C, C2/4 cells were transfected with 0.3 μg of HES1-luc or HES5-luc and 0.005 μg pRL-EF. Twenty-four hours later, the culture medium was changed to serum-free Dulbecco’s modified Eagle’s medium containing different amounts of the NOV protein produced and secreted by High Five cells. After 6 h of incubation, luciferase activity assay was conducted. Enhancement of HES1 and HES5 promoter activities was observed. RD, relative density.
**DISCUSSION**

Notch is a multimodal signal protein that has 36 EGF motifs and Lin/Notch repeats in the extracellular region and CDC10/ankyrin repeats and other motifs including nuclear localization signals in the intracellular region. On ligand binding, the intracellular domain of Notch is cleaved and translocates to the nucleus, where it associates with the transcription repressor CBF1 and consequently up-regulates the transcription factors such as *HES1* and *HES5* (42, 48–50). The Notch signaling system is supposed to be controlled by various factors, most of which that have been identified thus far are molecules that directly or indirectly interact with its intracellular region and participate in the signal transduction (51). As for molecules that modulate Notch signaling through interaction with the extracellular region of Notch, only *Drosophila* Wingless and Scabrous have currently been reported apart from the ligands (Delta, Serrate) (52, 53). Because NOV appears to have no invertebrate orthologues, its participation in Notch signaling is probably unique to vertebrate development.

The CT domain is predicted to form two twisted antiparallel pairs of β-strands with three disulfide bonds (12, 54–56). Although its amino acid sequences are poorly conserved, the location of their cysteine residues is well conserved, and the common structure is shared by several growth factors such as TGF-β, NGF, PDGF, and von Willebrand factor (12, 54). The CT domain is thought to mediate protein dimerization, and NOV, unlike CTGF, was suggested to be dimerized by this domain (29, 57). Because some of the receptor binding properties of TGF-β, NGF, and PDGF are within variable regions of the CT domain, it is thought that the CT domain regulates both dimerization and receptor binding (58, 59). Our results indicated that the CT domain of NOV is necessary for association with Notch1. Another example that the CT domain of NOV is used for association with other proteins is fibulin-1C, an extracellular matrix protein that mediates cell adhesion (29). Interestingly, the binding domain of fibulin-1C with NOV also contains six EGF motifs. Furthermore, Delta1 and Serrate1, which have 9 and 16 EGF motifs, respectively, showed a capability to associate with NOV, although it was much weaker than NOV-Notch1 association. CTGF, Cyr61, and NOV interact with in-
tigrin complex (60, 61); recently Takagi et al. (62) pointed out that the cysteine rich region of the integrin β-subunits contains EGF-like modules. These observations raise the possibility that CCN family members including NOV may associate with a broad range of proteins that contain EGF motifs.

A cell aggregation assay indicated that the region containing the 11th and 12th EGF motifs of Notch is the binding site of ligands (63, 64). The 11th and 12th EGF motifs of Notch are predicted to be calcium-binding EGF motifs, and the associations of Notch and its ligands have been shown to be Ca\textsuperscript{2+}-dependent (64), implying that the EGF motif conformation, which is maintained by an interdomain linkage in the presence of Ca\textsuperscript{2+}, is important for ligand binding (39, 40, 65). NOV-Notch association was Ca\textsuperscript{2+}-independent, implying that NOV binds to the non-calcium-binding EGF modules of Notch and may not interfere with the Delta (or Serrate)-Notch interaction. However, these two EGF motifs are not sufficient for Notch signal stimulation. In Drosophila, loss of the remaining extracellular sequence leads to impaired development, and several mutants with a single amino acid substitution in the other EGF motifs showed lethal phenotypes due to aberrant Notch function (66–69). The role of the EGF motifs other than the 11th and 12th remains unknown, but because most of these mutations seem to alter the structure of the EGF repeats, these facts suggest that interaction with the ligands is not only mediated by local protein structure but also affected by the whole conformation of the EGF repeats. A subtle change in conformation may affect the ligand-receptor interaction and modulate the signal receptivity, which would provide a rationale for the effect of NOV on the Notch signaling pathway. Or it may be that NOV enhances the receptivity of Notch by facilitating its multimerization. This explanation seems plausible, considering that NOV can also form a homodimer as well as associate with Notch. Further analysis will be necessary to elucidate these hypotheses.

The Notch signaling system plays an essential role in determining cell fate, and generally its signal keeps multipotent progenitor cells at an uncommitted state. So-called lateral inhibition, which prevents an ectodermal precursor cell from taking neural fate in Drosophila, is a well-known example. In vertebrates, cell culture studies demonstrated that the expression of constitutively active Notch inhibits the differentiation of several cell lines, i.e. neural, hematopoietic, or myogenic cells (70–73). These results were frequently interpreted to mean that Notch signaling is essential to maintain the progenitor cells in an immature proliferative state. This interpretation is supported by several lines of evidence. However, in myogenesis, an in vivo study (74) revealed that Notch expression in myoblasts is restricted to the postmitotic cells and that Notch signaling did not inhibit their exit from the cell cycle, implying that the role of Notch signaling in myogenesis is not to maintain the progenitor cells but to participate in later differentiation events. In the present study, we used C2/4 myogenic cells, a subclone of the C2C12 mesenchymal cell line (43, 44). Notch signaling is crucial for the suppressive regulation of C2/12 differentiation, a process that is executed through two independent Notch signaling cascades (73). One is a cell-type-specific CBF1-dependent pathway that transactivates HES1 and suppresses myogenic transcription factors such as MyoD. The other signaling cascade is independent of CBF1 and regulates general steps of differentiation. We demonstrated that elevated expression of NOV inhibits the differentiation of C2/4, with down-regulation of MyoD and myogenin, although the level of Notch1 expression was not altered. Our results suggest that the positive effect of NOV on Notch signaling promotes its inhibitory effect on myogenic differentiation via the CBF1-dependent Notch-HES1-MyoD pathway. Although HES1 and HES5 play similar roles in neurogenesis (75, 76), it is not clear whether HES5 is required for myogenesis as is HES1. Modest activation of HES5 promoter in C2/4 cells might implicate its relatively small contribution to myogenesis.

Chen et al. (61) showed that CTGF and Cyr61 bind to integrin and activates the focal adhesion kinase (FAK) signaling cascade. We found that NOV can cause tyrosine phosphorylation of FAK, and therefore there is a possibility that NOV can partially substitute for the function of CTGF and activate the FAK signaling cascade. FAK plays a central role in notochord and somite morphogenesis, mediating their boundary formation and maintenance. Recently, Henry et al. (77) showed that Xenopus FAK is expressed in notochord and the notochord-somite boundary and that phosphorylated FAK protein is seen both at the notochord-somite boundary and at inter somitic boundaries. Ectopic activation of Notch signaling by a constitutively activated form of Su(H)/CBF1 mRNA injection resulted in nonsegmental expression of FAK and disrupted the normal somite segmentation (77), suggesting that the Notch signaling pathway may regulate the FAK expression. These results implicate another possible cross-talk between NOV and Notch in mediating somite segmentation through FAK signaling cascade.

In conclusion, we have demonstrated a novel interaction between the CT domain of NOV and the EGF repeats of Notch, which positively regulates the Notch signaling pathway and suppresses myoblast differentiation. Low affinity bindings of NOV with the other EGF motifs have also been observed, which raises the question of whether NOV or the other CCN members interact with many different EGF motif-containing molecules. The results reported in this manuscript reinforce the hypothesis (30) that a broad spectrum of possible interacting factors may explain the general property of the CCN members, which exhibits various and sometimes paradoxical functions depending on the cell types and conditions.

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