Tage4/Nectin-like Molecule-5 Heterophilically \textit{trans}-Interacts with Cell Adhesion Molecule Nectin-3 and Enhances Cell Migration*

Wataru Ikeda‡, Shigeki Kakunaga‡, Shinsuke Itoh‡, Tatsushi Shingai‡, Kyoji Takekuni‡, Keiko Satoh‡, Yoko Inoue‡, Akiko Hamaguchi‡, Koji Morimoto‡, Masakazu Takeuchi‡, Toshio Imai§, and Yoshimi Takai‡‡

From the \textit{Department of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine/Faculty of Medicine, Suita 565-0871, Japan and the SKAN Research Institute Inc., 93 Chudoji-Awatamachi, Shimogyo-ku, Kyoto 600-8815, Japan}

Malignant transformation of cells causes disruption of cell-cell adhesion, enhancement of cell motility, and invasion into surrounding tissues. Nectins have both homotypic and heterotypic cell-cell adhesion activities and organize adherens junctions in cooperation with cadherins. We examined here whether Tage4, which was originally identified to be a gene overexpressed in colon carcinoma and has a domain structure similar to those of nectins, is involved in cell adhesion and/or migration. Tage4 heterophilically \textit{trans}-interacted with nectin-3, but not homophilically with Tage4. Expression of Tage4 was markedly elevated in NIH3T3 cells transformed by an oncogenic Ki-Ras (V12Ras-NIH3T3 cells) as compared with that of wild-type NIH3T3 cells. \textit{trans}-Interaction of Tage4 with nectin-3 enhanced motility of V12Ras-NIH3T3 cells. Tage4 did not bind afadin, a nectin- and actin filament-binding protein that connects nectins to the actin cytoskeleton and cadherins through catenins. Thus, Tage4 heterophilically \textit{trans}-interacts with nectin-3 and regulates cell migration. Tage4 is tentatively renamed here nectin-like molecule-5 (necl-5) on the basis of its function and domain structure similar to those of nectins.

In multicellular organisms, cell adhesion and migration are critical for many events, including tissue patterning, morphogenesis, and maintenance of normal tissues (1–3). They also play roles in malignant transformation of cells (4). Adhesion and migration of non-transformed normal cells are dynamic and well regulated (2). Cells disrupt cell-cell adhesion and start to migrate in response to extracellular cues, such as growth factors, cytokines, and extracellular matrix molecules (4). When migrating cells contact other cells, they stop migration and proliferation and adhere to each other to become confluent (5, 6). This phenomenon is known for a long time as contact inhibition of cell movement and proliferation. Transformation of cells causes disruption of cell-cell adhesion, increase of cell motility, and loss of contact inhibition of cell movement and proliferation, eventually leading the transformed cells to invasion into surrounding tissues and metastasis to other organs (4, 7). However, molecular mechanisms underlying these physiological or pathological processes are not fully understood.

Cell-cell adherens junctions (AJs)\textsuperscript{1} play major roles in cell-cell adhesion in fibroblasts and epithelial cells (1, 2). Cadherins are key Ca\textsuperscript{2+}-dependent cell-cell adhesion molecules at AJs (1, 2). Cadherins are associated with the actin cytoskeleton through peripheral membrane proteins, including \(\alpha\) and \(\beta\)-catenins, in fibroblasts and epithelial cells (1). This association strengthens the cell-cell adhesion activity of cadherins (1). Nectins and afadin constitute another cell-cell adhesion unit that localizes at cell-cell AJs and regulates organization of AJs in cooperation with cadherins in fibroblasts and epithelial cells (8). Nectins are Ca\textsuperscript{2+}-independent Ig-like cell-cell adhesion molecules. Afadin is a nectin- and actin filament-binding protein that connects nectins to the actin cytoskeleton. Nectins comprise a family of four members, nectin-1, -2, -3, and -4, each of which has two or three splicing variants. Nectins have one extracellular region with three Ig-like loops, one transmembrane region, and one cytoplasmic region. All nectins except nectin-4 have a C-terminal conserved motif of four amino acids (aa) residues, which interacts with the PDZ domain of afadin. Nectin-4 does not have this motif but binds afadin. Each nectin forms homo-	extit{cis}-dimers, followed by the formation of homo-	extit{trans}-dimers, causing cell-cell adhesion. Nectin-3 further heterophilically \textit{trans}-interacts with nectin-1 or -2 and the adhesion activity of these heterophilic \textit{trans}-interactions is stronger than that of the homophilic \textit{trans}-interactions. Nectin-4 also heterophilically \textit{trans}-interacts with nectin-1.

Five or six molecules having one extracellular region with three Ig-like loops, one transmembrane region, and one cytoplasmic region have thus far been identified (Table I) (9–19).

\textsuperscript{1} The abbreviations used are: AJ, adherens junction; aa, amino acid(s); necl, nectin-like molecule; V12Ras-NIH3T3 cells, NIH3T3 cells stably expressing V12Ki-Ras; SEAP, secreted alkaline phosphatase; Neap, the extracellular fragment of necl fused to SEAP; Nef, the extracellular fragment of nectin fused to the human IgG Fc; nectin-L cells, L cells stably expressing full-length human nectin-1a; nectin-2L cells, L cells stably expressing full-length mouse nectin-2c; nectin-3L cells, L cells stably expressing full-length mouse nectin-3o; non-tagged-nect-5L cells, L cells stably expressing full-length nectin-5L cells; L cells stably expressing FLAG-nect-5L; nectin-5L cells, L cells stably expressing FLAG-nect-5L; nectin-5L-3EC-L cells, L cells stably expressing FLAG-nect-5L-3EC-L; Ab, antibody; Leu, the extracellular fragment of nectin fused to the human IgG Fc; GST, glutathione S-transferase; GST-nect-5L, the cytoplasmic tail of nectin fused to GST; Leu, the extracellular fragment of nectin fused to SEAP; mAb, monoclonal Ab; pAb, polyclonal antibody; DiI, 1,1'-dioctadecyl-3,3',3'-tetramethyl-indocarbocyanine perchlorate; EL cells, L cells stably expressing full-length E-cadherin.

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\textsuperscript{†} To whom correspondence should be addressed: Dept. of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine, Osaka University Graduate School of Medicine, Osaka, Japan. Tel.: 81-6-6879-3410; Fax: 81-6-6879-3419; E-mail: ytakai@molbio.med.osaka-u.ac.jp.

\textsuperscript{‡} To whom correspondence should be addressed: Dept. of Molecular Biology and Biochemistry, Osaka University Graduate School of Medicine, Osaka University Graduate School of Medicine, Osaka, Japan and the SKAN Research Institute Inc., 93 Chudoji-Awatamachi, Shimogyo-ku, Kyoto 600-8815, Japan.
We tentatively name here these molecules nectin-like molecules (necls) on the basis of their domain structures similar to those of nectins (see "Discussion"). Of these necls, Tage4 was originally identified to be a gene overexpressed in rat and mouse colon carcinoma (16, 17). Northern blot analysis has revealed that Tage4 is expressed in normal adult rat and mouse tissues to small extents (16, 17), but its function remains unknown, except that it mediates entry of porcine pseudorabies virus and bovine herpesvirus 1 (20). We have studied here the function of Tage4 and revealed that Tage4 heterophilically trans-interacts with nectin-3 and regulates cell migration. Tage4 is tentatively re-named here necl-5 on the basis of its function and phylogenetic tree of nectins and necls (Fig. 1) (see "Discussion").

**EXPERIMENTAL PROCEDURES**

**Molecular Cloning of Mouse Necl-5 cDNA**—The cDNA of mouse Tage4/necl-5 was originally isolated by reverse transcriptase-PCR from the C26 mouse colon carcinoma cell line (DDBJ/GenBank™/EBI accession number MMU38360 (17). This cell line was derived from BALB/c mice. Because we generally use necls derived from C57BL/6 mice, we re-cloned the Tage4/necl-5 cDNA derived from C57BL/6 mice. We searched in the DNA data base and found one sequence similar to that of Tage4/necl-5 (DDBJ/GenBank™/EBI accession number BC013673). We performed reverse transcriptase-PCR from mouse brain total RNA of C57BL/6 mice on the basis of BC013673. The new sequence of Tage4/necl-5 showed 93% nucleotide identity to that of the original one. The C-terminal half was identical, but the N-terminal half was slightly different. The new sequence was identical to BC013673 except for the exchange of a single nucleotide from cytosine to adenine, at position 854 (open reading frame). The reason for this difference is not known, but may be due to the different strains of mice. We confirmed that the re-cloned Tage4/necl-5 cDNA sequence was full length.

**Construction of Plasmids**—Expression vectors were constructed in pFLAG-CMV1 (Sigma), pCAGIPuro (21), pCAGIZeo (22), pGEX4T-1 (Amersham Biosciences), pGBD-C1 (23), pFastBac1-Msp-Fc-necl-5-EC (24), and pDREF-SEAP(His)6-Hyg (25). Constructs of necl-5 contained the following aa: pCAGIZeo-necl-5, aa 1–409 (full-length); pFLAG-CMV1-necl-5, aa 30–409 (deleting the signal peptide); pCAGIPure-FLAG-necl-5, aa 30–409 (including the preprotrypsin signal peptide); pFLAG-CMV1-necl-5-EC, aa 335–409 (deleting the extracellular region); pCAGIZeo-FLAG-necl-5-EC, aa 335–409 (including the preprotrypsin signal peptide); pGEX4T-1-necl-5-CP, aa 371–409 (the cytoplasmic region); pGBD-C1-necl-5-EC, aa 335–409 (deleting the extracellular region); pFastBac1-Msp-Fc-necl-5-EC, aa 30–347 (the extracellular region lacking the signal peptide); and pDREF-SEAP(His)5-Hyg-necl-5-EC, aa 1–347 (the extracellular region). To express the extracellular fragment of necl-5 we used three different vectors: (i) the SEAP reporter vector pDREF-SEAP(His)5-Hyg, which expresses a secretable alkaline phosphatase, (ii) the GST fusion protein pGEX4T-1-necl-5-CP, which expresses a phosphorylatable fusion protein, and (iii) the IgG Fc fusion protein pGBD-C1-necl-5-EC, which expresses an extracellular fragment of necl-5 fused to the human IgG Fc (Nef-3), and (iv) the SEAP reporter vector pDREF-SEAP(His)5-Hyg-necl-5-EC, which expresses a secretable alkaline phosphatase.

**Cell Culture and Establishment of Transfectants**—L and MTD-1A cells were kindly supplied by Dr. S. Tsukita (Kyoto University, Kyoto, Japan). MDCK cells were kindly supplied by Dr. W. Birchmeier (Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany). V12Ras-NIH3T3 cells were prepared as described (27). L, MTD-1A, and MDCK cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% FCS. NIH3T3 and V12Ras-NIH3T3 cells were maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% calf serum. L cell lines stably expressing full-length human nectin-1a, necl-5, necl-3, necl-2, necl-1, necl-4, necl-6, Tage4, and necl-6, PVR/CD155.

**Interaction of Necl-5/Tage4 with Nectin-3 and Cell Migration**

We performed reverse transcriptase-PCR from mouse brain total RNA (28) and successfully re-cloned the Tage4/necl-5 cDNA derived from C57BL/6 mice. We re-cloned the Tage4/necl-5 cDNA derived from C57BL/6 mice. We confirmed that the re-cloned Tage4/necl-5 cDNA sequence was full length.
full-length mouse nectin-2a, or full-length mouse nectin-3a (nectin-1-L, -2-L, or -3-L cells, respectively) were prepared as described previously (21, 24, 28). As L cell line stably expressing full-length nectin-5 (non-tagged-necl-5-L cells), FLAG-necl-5 (necl-5-L cells), or FLAG-necl-5-ΔEC (necl-5-ΔEC-L cells) was obtained by transfection with pcAGIze-necl-5, pcAGI Puro-FLAG-necl-5, or pcAGI Zeo-FLAG-necl-5-ΔEC, respectively, using LipofectAMINE PLUS reagent (Invitrogen). We mostly used necl-5-L cells (FLAG-tagged necl-5) in the present study, but the essentially similar results were obtained with non-tagged-necl-5-L cells (data not shown).

**Antibodies**—A rat anti-necl-5 monoclonal antibody (mAb) #1A8-8 was raised against the extracellular fragment of necl-5 (aa 30–347) fused to the human IgG Fc (Lef-5). A rabbit antiserum against necl-5 was raised against the extracellular fragment of necl-5 fused to SEAP (LeaP 5) and immobilized Nef-3 or LeaP-5. The Fab fragment of anti-human IgG Fc polyclonal Ab (pAb) was immobilized at a concentration of about 4800 resonance units (4.8 ng/mm²) to the sensor chip surface by the amine-coupling method. Nef-3 or LeaP-5 was immobilized at a concentration of about 500 resonance units to the sensor chip via the anti-human IgG Fc Ab. Neap-1, Neap-2, or LeaP-5 was then diluted in HBS-EP buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 3 mM EDTA, 0.005% Tween 20; BIAcore) to 40 nM and injected at a flow rate of 20 μl/min at 25°C for 210 s. Both an association rate constant $k_a$ (s⁻¹) and a dissociation rate constant $k_d$ (s⁻¹) were obtained using the BIAevaluation software version 3.2 (BIAcore), and the dissociation constant ($K_D = k_d/k_a$) was derived from the two deduced rate constants.

**Intercellular Motility**—Intercellular motility assay was done as described previously (29). Briefly, the cells were labeled with 11'-diodoteadecyl-3,3',3'-tetramethylindocarbocyanine perchlorate (DiI), and the 1 × 10⁶ labeled cells were seeded on a confluent culture of 2 × 10⁶ unlabeled nectin-3-L cells in a 24-well dish. After 36 or 48 h of culture, four sister cells that seemed to be derived from one seeded cell were examined by fluorescence microscopy. In the experiments using the anti-necl-5 mAb, this mAb was added at a final concentration of 50 μg/ml in the medium. When a cell line A was seeded on a confluent culture of a cell line B, we designated the experiment as A/B analysis. For quantification of intercellular motility, intercellular distances of all cell combinations between four sister cells were measured and summed as Da. As a control experiment, the labeled cells were seeded on dishes in the absence of a cell layer. In this case, the intercellular distances were summed as Dd. The degree of intercellular motility was represented as DA/Dd. At least 24 independent samples were picked up to determine Da or Dd for each cell line.

**RESULTS**

**Heterophilic trans-Interaction of Necl-5 with Nectin-3**—We first examined by the aggregation assay using necl-5-L cells whether necl-5 has cell-cell adhesion activity. In wild-type L cells, nectin-1 and -2, but not cadherin or nectin-3, are expressed as estimated by Western blotting (21, 24, 26). Expression of necl-5 was not detected in wild-type L cells by Western blotting using any Abs, which recognized exogenously expressed necl-5 (see Fig. 5). Wild-type L cells did not form visible cell aggregates as described previously (28) (Fig. 2A). Necl-5-L cells did not form visible cell aggregates, either (Fig. 2B). These results indicate that necl-5 does not homophilically trans-interact with necl-5, causing no homophilic cell-cell adhesion.

We then examined whether necl-5 has heterophilic cell-cell adhesion activity with other nectins. Necl-5-L cells were mixed with nectin-1-L, -2-L, or -3-L cells followed by the aggregation assay. Nectin-3-L cells formed small aggregates in the absence of necl-5-L cells as described previously (29) (Fig. 2C), but formed relatively big aggregates with necl-5-L cells (Fig. 2D, Da–Dc). This aggregate was similarly formed even in the absence of Ca²⁺ (data not shown). The size of the aggregates formed between necl-5-L and nectin-3-L cells were about 20% that of the aggregates formed between nectin-1-L and -3-L cells, which formed the biggest aggregates among various combinations of nectins thus far examined (32) (see Fig. 7, Aa and Ba). Necl-5-L cells did not form mixed aggregates with nectin-1-L or -2-L cells (Fig. 2, Ea–Ec and Fa–Fc). Small aggregates observed by necl-5-L cells or nectin-2-L cells by themselves as described (28) (Fig. 2, Ea, Ec, Fa, and Fc). These results indicate that necl-5 heterophilically trans-interacts selectively with nectin-3 in a Ca²⁺-independent manner, causing
nectin-2 or -3 bound it (data not shown). We estimated by yeast two-hybrid assay, co-immunoprecipitation for binding to the PDZ domain (data not shown). We examined necl-5 does not have a C-terminal consensus motif with four aa of necl-5 showed 28–42% aa identity to that of nectins, but any band was not detected in cultured cell lines, including L (Fig. 5), MTD-1A (data not shown), and MDCK cells (data not shown), but two faint bands were detected in NIH3T3 cells (Fig. 5). Stage4 was originally isolated from rat and mouse colon carcinoma (16, 17). We therefore examined the expression of necl-5 in V12Ras-NIH3T3 cells. Expression of necl-5 was markedly elevated in the transformed cells as compared with that of the wild-type cells (Fig. 5).

**Enhancement of Motility of V12Ras-NIH3T3 and Necl-5-L Cells by trans-Interaction of Necl-5 with Nectin-3.—** We finally studied the role of the trans-interaction of necl-5 with nectin-3 on motility of V12Ras-NIH3T3 cells by using intercellular motility assay, because transformed cells show generally enhanced migration activity (4). Necl-5-L and necl-5-ΔEC-L cells were used as control cells. Necl-5-ΔEC-L cells were L cells expressing necl-5, of which extracellular region except the juxtamembrane 13 aa were deleted. In this assay, cell motility in the absence of the anti-necl-5 mAb; Ab and Bb, in the presence of the anti-necl-5 mAb. Bars, 100 μm. The results shown are representative of three independent experiments.

**Elevated Expression of Necl-5 in V12Ras-NIH3T3 Cells—** We then examined the tissue distribution of necl-5 in mouse by Western blotting, but the significant immunoreactive band was not detected in any normal tissue examined, including heart, brain, spleen, lung, liver, kidney, skeletal muscle, and testis (data not shown), consistent with the earlier observation (17). Any band was not detected in cultured cell lines, including L (Fig. 5), MTD-1A (data not shown), and MDCK cells (data not shown), but two faint bands were detected in NIH3T3 cells (Fig. 5). Stage4 was originally isolated from rat and mouse colon carcinoma (16, 17). We therefore examined the expression of necl-5 in V12Ras-NIH3T3 cells. Expression of necl-5 was markedly elevated in the transformed cells as compared with that of the wild-type cells (Fig. 5).

**Specific Inhibition of the Interaction of Necl-5 with Nectin-3 by the Anti-necl-5 mAb.** The inhibitory activity of the anti-necl-5 mAb was assayed by the cell aggregation assay. A, necl-5-L and nectin-3-L cells; B, nectin-1-L and nectin-3-L cells. Aa and Bb, in the absence of the anti-necl-5 mAb; Ab and Bb, in the presence of the anti-necl-5 mAb. Bars, 100 μm. The results shown are representative of three independent experiments.

**Interaction of Necl-5/Tage4 with Nectin-3 and Cell Migration**

**FIG. 6.** Enhancement of motility of V12Ras-NIH3T3 and necl-5-L cells by trans-interaction of necl-5 with nectin-3. A, inter-nectin-3-L-cellular motility. Panels a, V12Ras-NIH3T3/nectin-3-L analysis; panels b, necl-5-L/nectin-3-L analysis; and panels c, necl-5-ΔEC-L/nectin-3-L analysis. Panels a1, b1, and c1, on the cell layer; panels a2, b2, and c2, on the dish; panels a3, b3, and c3, on the cell layer in the presence of the anti-necl-5 mAb; and panels a4, b4, and c4, on the dish in the presence of the anti-necl-5 mAb. The labeled four sister cells were indicated as red images by the fluorescence microscopy. In panels a1, a3, b1, b3, c1, and c3, these images were superimposed on the phase contrast image of a confluent cell layer, and the labeled four sister cells were indicated as white arrows. Bars, 100 μm. B, quantitative analysis of the intercellular motility. The average value of intercellular motility is shown here. At least 24 independent samples were picked up to determine the intercellular motility index for each analysis.

**FIG. 7.** Specific inhibition of the interaction of necl-5 with nectin-3 by the anti-necl-5 mAb. The inhibitory activity of the anti-necl-5 mAb was assayed by the cell aggregation assay. A, necl-5-L and nectin-3-L cells; B, nectin-1-L and nectin-3-L cells. Aa and Bb, in the absence of the anti-necl-5 mAb; Ab and Bb, in the presence of the anti-necl-5 mAb. Bars, 100 μm. The results shown are representative of three independent experiments.
cells scattered on a confluent culture of nectin-3-L cells more actively than on the dish (Fig. 6, Aa1, Aa2, and B). The scattering of V12Ras-NIH3T3 cells on nectin-3-L cells was inhibited by the anti-necl-5 mAb, whereas the scattering on the dish was not affected by this mAb (Fig. 6, Aa3, Aa4, and B). The anti-necl-5 mAb inhibited the interaction of necl-5 with nectin-3 as estimated by the aggregation assay using necl-5-L and nectin-3-L cells (Fig. 7, Aa and Aa6). This mAb did not affect the interaction of necl-5-L with nectin-3-L (Fig. 7, Bb and Bb6). Necl-5-L cells labeled with DiI were seeded on a confluent culture of non-labeled nectin-3-L cells, and after 48 h (twice the doubling time), the cell scatter property was similarly analyzed. Necl-5-L cells scattered on a confluent culture of nectin-3-L cells more actively than on the dish (Fig. 6, Ab1, Ab2, and B). The scattering of necl-5-L cells on nectin-3-L cells was inhibited by the anti-necl-5 mAb, whereas the scattering of necl-5-L cells on the dish was not affected by this mAb (Fig. 6, Ab3, Ab4, and B). Necl-5-ΔEC-L cells labeled with DiI scattered on a confluent culture of nectin-3-L cells less actively than on the dish (Fig. 6, Ac1, Ac2, and B). Necl-5-ΔEC-L cells did not adhere to nectin-3-L cells as estimated by the aggregation assay (data not shown). The scattering of necl-5-ΔEC-L cells in the presence or absence of nectin-3-L cells was not affected by the anti-necl-5 mAb (Fig. 6, Ac3, Ac4, and B). These results indicate that the trans-interaction of necl-5 with nectin-3 enhances motility of V12Ras-NIH3T3 and necl-5-L cells.

**DISCUSSION**

We have shown here that necl-5 does not homophilically trans-interact with necl-5, but heterophilically trans-interacts selectively with nectin-3, causing cell-cell adhesion. This property of necl-5 is quite different from that of nectins which both homophilically and heterophilically trans-interact (8). We have previously proposed that nectins are involved in the formation of AJs in cooperation with E-cadherin, on the basis of the observations that the trans-interaction of nectins recruits E-cadherin to the nectin-based cell-cell adhesion sites, resulting in formation of AJs, and that the disruption of this trans-interaction of nectins by their antagonists impairs the formation of E-cadherin-based AJs (8). The association of nectins and E-cadherin at AJs is mediated through afadin and α-catenin (8). We have shown here that necl-5 does not bind afadin. The inability of necl-5 to bind afadin suggests that necl-5 has no potency to recruit cadherins to the cell-cell adhesion site formed by the trans-interaction of necl-5 with nectin-3 and is not involved in the formation of AJs.

We have shown here that the heterophilic trans-interaction of necl-5 with nectin-3 rather enhances motility of V12Ras-NIH3T3 and necl-5-L cells. It has previously been reported that L cells stably expressing full-length E-cadherin (EL cells) shows inter-EL-cellular EL cell motility (29, 33). The mechanism of this intercellular motility of EL cells is not clear, but it has been suggested that dynamic attachment of EL cells to neighboring EL cells and dynamic detachment of EL cells from neighboring EL cells are necessary for the motility of EL cells (29, 33). The mechanism of intercellular motility of V12Ras-NIH3T3 and necl-5-L cells is not known, either, but may be analogous to that of EL cells.

Transformation of cells increases cell motility, causing invasion into surrounding tissues. Since expression of necl-5 is elevated by transformation as shown here and described previously (16, 17), this elevation of necl-5 may be at least partly responsible for the enhanced cell motility and invasion of transformed cells. Intercellular motility is observed in vivo in the process of morphogenetic rearrangement of cells in embryonic tissues (2, 3). It remains unknown what kind of the cells express necl-5 in embryonic tissues, but if necl-5 is expressed in rapidly migrating cells, such as mesenchymal cells, the dynamic trans-interaction of necl-5 with nectin-3 may also play a role in their intercellular motility. Further studies are necessary for establishing the physiological and pathologcal roles of necl-5 in these processes.

We lastly discuss about other necls which have thus far been identified in addition to necl-5/Tage4 (Table I). Five or six necls including necl-5 have been identified but have many nomenclatures. We propose here that a group of proteins with structures similar to those of nectins but without ability to directly bind afadin are called nectin-like molecules (necls). NECL1/TSL1/SynCAM3, NECL2/IGSF4/RA175/SgIGSF/TSLC1/Syn-
CAM1, NECL3/similar to NECL3/SynCAM2, and TSL2/SynCAM4 have a C-terminal consensus motif with four aa for binding to PDZ domains, and these cytoplasmic regions show high similarity. Necl-5/Tage4 or PVR/CD155 does not have this motif. Our analysis has revealed that NECL1/TSL1/SynCAM3 or NECL2/IGSF4/RAI175/SgIGSF/TSLC1/SynCAM1 as well as necl-5/Tage4 does not bind afadin as estimated by yeast two-hybrid assay, co-immunoprecipitation assay, and affinity chromatography (data not shown). It remains to be examined whether NECL3/similar to NECL3/SynCAM2, TSL2/SynCAM4, or PVR/CD155 binds afadin. On the assumption that all of these molecules are called necls. Then, we propose the following nomenclatures according to the phylogenetic tree shown in Fig. 1: necl-1 for NECL1/TSLL1/SynCAM3, necl-2 for NECL2/IGSF4/RAI175/SgIGSF/TSLC1/SynCAM1, necl-3 for NECL3/similar to NECL3/SynCAM2, necl-4 for TSL2/SynCAM4, necl-5 for Tage4, and necl-6 for PVR/CD155. The necl-5/Tage4 gene may be an ortholog of the necl-6/PVR/CD155 gene (20), but the phylogenetic tree of necl-5/Tage4 and necl-6/PVR/CD155 are derived from the same or different ancestor gene. Therefore, we reserve the conclusion that these three proteins do not directly bind afadin, we propose that all of these molecules are called necls.

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Note Added in Proof—Mueller and Wimmer (Mueller, S., and Wimmer, E., 2003) J. Biol. Chem. 278, 6855–6865 demonstrated the heterophilic trans-interactions of PVR/CD155 and Tage4/necl-5 with nectin-3, and they furthermore showed that PVR/CD155 colocalized with integrin α,β3, Reymond et al. (Reymond, N., Fabre, S., Lecocq, E., Adelaide, J., Dubreuil, P., and Lopez, M., 2001) J. Biol. Chem. 276, 43205–43215 previously claimed the PVR/CD155 bound nectin-3, although no experimental evidence for this interaction was presented.

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