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Review

Interactions of the cell adhesion molecule nectin with transmembrane and peripheral membrane proteins for pleiotropic functions

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Abstract. Cell adhesion molecules (CAMs) have been implicated in the control of a wide variety of cellular processes, such as cell adhesion, polarization, survival, movement, and proliferation. Nectins have emerged as immunoglobulin-like CAMs that participate in calcium-independent cell-cell adhesion by homophilic and heterophilic *trans*-interactions with nectins and nectin-like molecules. Nectin-based cell-cell adhesion exerts its function independently or in cooperation with other CAMs including cadherins and is essential for the formation of intercellular junctions, including adherens junctions, tight junctions, and puncta adherentia junctions. Nectins *cis*-interact with integrin $\alpha_v\beta_3$ and platelet-derived growth factor receptor and facilitate their signals to regulate the formation and integrity of intercellular junctions and cell survival. Nectins intracellularly associate with peripheral membrane proteins, including afadin and Par-3. This review focuses on recent progress in understanding the interactions of nectins with other transmembrane and peripheral membrane proteins to exert pleiotropic functions.

Keywords. Cell adhesion molecule, nectin, cadherin, integrin, PDGF, Necl-5.

Introduction

Cell adhesion molecules (CAMs) have been implicated in the regulation of a wide variety of fundamental cellular processes, not only cell adhesion, but also cell polarization, survival, movement, and proliferation. Nectins have emerged as Ca^{2+}-independent immunoglobulin (Ig)-like CAMs with three extracellular Ig-like domains, a single transmembrane region, and a cytoplasmic tail (Fig. 1a). Nectins consist of a family with four members (nectin-1 to nectin-4) and each member has splicing variants, such as nectin-1α, nectin-1β, nectin-1γ, nectin-2α, nectin-2δ, nectin-3α, nectin-3β, and nectin-3γ, together with two splicing isoforms of nectin-4 whose names have yet to be determined [1–5]. Nectin-1, nectin-2, and nectin-3 are widely distributed in embryos and adult tissues, whereas nectin-4 is expressed mainly in placenta in humans, despite a broad expression in mouse tissues [1]. Nectins initially form homophilic *cis*-dimers through the second Ig-like domain on the cell surface

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and these dimers then *trans*-interact to form homophilic and/or heterophilic *trans*-dimers through the first Ig-like domain at cell-cell adhesion sites. All nectins are able to form homophilic *trans*-interactions; that is, a subset of nectin on one cell binds to the same molecule on other cells (Fig. 2a). In addition, nectins can heterophilically *trans*-interact with other subsets of nectins; nectin-1 *trans*-interacts with nectin-3 and nectin-4, while nectin-2 *trans*-interacts with nectin-3 (Fig. 2a). Heterophilic *trans*-interactions of nectins exhibit higher affinity than homophilic *trans*-interactions, and the difference in affinity between heterophilic and homophilic *trans*-interactions actually determines the type of cell-cell adhesion. Nectin-based cell-cell adhesion plays an essential role in the formation of intercellular junctions, such as adherens junctions (AJs), tight junctions (TJs), and puncta adherentia junctions, and exerts its function independently or in cooperation with other CAMs including cadherins. Nectin-based cell-cell adhesion initiates AJ formation in epithelial cells and fibroblasts and puncta adherentia junction formation in neurons in cooperation with cadherins, while it controls TJ formation in epithelial cells in cooperation with TJ transmembrane proteins, such as junctional adhesion molecules (JAMs), claudins, and occludin [6, 7]. Nectins directly interact with the actin filament (F-actin)-binding protein afadin and the cell polarity protein Par-3 through the cytoplasmic tail (Fig. 1a) and also associate indirectly with a diverse set of peripheral membrane proteins, such as afadin DIL domain-interacting protein (ADIP), LIM domain only 7 (LMO7), ZO proteins, ponsin, α-catenin, $p120^{ctn}$, annexin II, and IQGAP1. Large complex formation resulting from interactions of nectins with these peripheral membrane proteins is critical for the formation and integrity of AJs and TJs.

Nectin-1 and nectin-3 interact in *cis* with integrin $\alpha_v\beta_3$, and these interactions between nectins and integrin $\alpha_v\beta_3$ facilitate the transduction of their signals to the cytoplasm to mediate the reorganization of the actin cytoskeleton required for the formation of AJs and TJs [8, 9]. In addition, nectin-3 *cis*-interacts with platelet-derived growth factor (PDGF) receptor (PDGFR) (Fig. 2b), thereby regulating cell survival signaling (unpublished data). On the other hand, nectin-3 *trans*-interacts with nectin-like molecule-5 (Necl-5), which is implicated in the regulation of cell movement and proliferation [6–11]. This review will focus on recent progress in understanding the interactions of nectins with other transmembrane and peripheral membrane proteins to exert pleiotropic functions. Other aspects of the biology of nectins have been comprehensively reviewed elsewhere [6–11].

Regulation of cell adhesion

Nectin-based cell-cell adhesion has emerged as the initial step of intercellular junction formation in epithelial cells and non-epithelial cells. Nectins homophilically and/or heterophilically *trans*-interact with nectins at cell-cell adhesion sites. It has been shown that nectins through their cytoplasmic tails possessing a PDZ domain-binding motif directly or indirectly interact with a variety of peripheral membrane proteins, and these interactions are implicated in the formation of intercellular junctions. For example, nectins directly bind the PDZ domain of afadin, which links nectins to the actin cytoskeleton; nectin-1, nectin-2, and nectin-3 bind afadin through a C-terminal conserved motif with four amino acids (Glu/Ala-X-Tyr-Val; X is any amino acid), whereas nectin-4, despite the absence of the C-terminal conserved motif, also binds afadin through its C terminus (Fig. 1a) [6–8, 12]. Afadin has multiple protein interaction domains, including two Ras association (RA) domains, a forkhead-associated (FHA) domain, a DIL domain, a PDZ domain, three proline-rich (PR) domains, and an F-actin-binding domain (Fig. 1a), and associates with a variety of cytoplasmic proteins, such as Rap1, ADIP, LMO7, ZO-1, ponsin, $p120^{ctn}$, and α-catenin [6–13]. ADIP and LMO7 connect afadin to α-actinin, while ponsin connects afadin to vinculin. ZO-1 binds TJ transmembrane proteins, such as JAMs, occludin, and claudins, besides afadin [14–16]. By virtue of the interactions with these proteins, nectins can associate with cadherins, key Ca^{2+}-dependent CAMs, and the associations between nectins and cadherins have been defined to be essential for the formation and integrity of cell-cell adhesion in epithelial cells, fibroblasts, and neurons [17, 18]. Nectin-1 and nectin-3 can *cis*-interact with integrin $\alpha_v\beta_3$ through their extracellular regions (Fig. 2b) [19]. The discovery of associations between nectins and integrin $\alpha_v\beta_3$ has provided a new perspective to our understanding of an association between cell-cell and cell-matrix junctions.

Implication of interactions of nectins with cadherins through the cytoplasmic tail in the formation of AJs

The function of nectins in intercellular junction formation has been best characterized in epithelial AJ formation [6–12]. Accumulating evidence indicates that nectins create the initial cell-cell adhesion and then recruit E-cadherin to the nectin-based cell-cell adhesion sites, forming AJs. Although the molecular mechanisms underlying the physical associations between nectins and E-cadherin are not completely understood, both afadin and α-catenin are essential for their interactions [6–12]. The interactions of

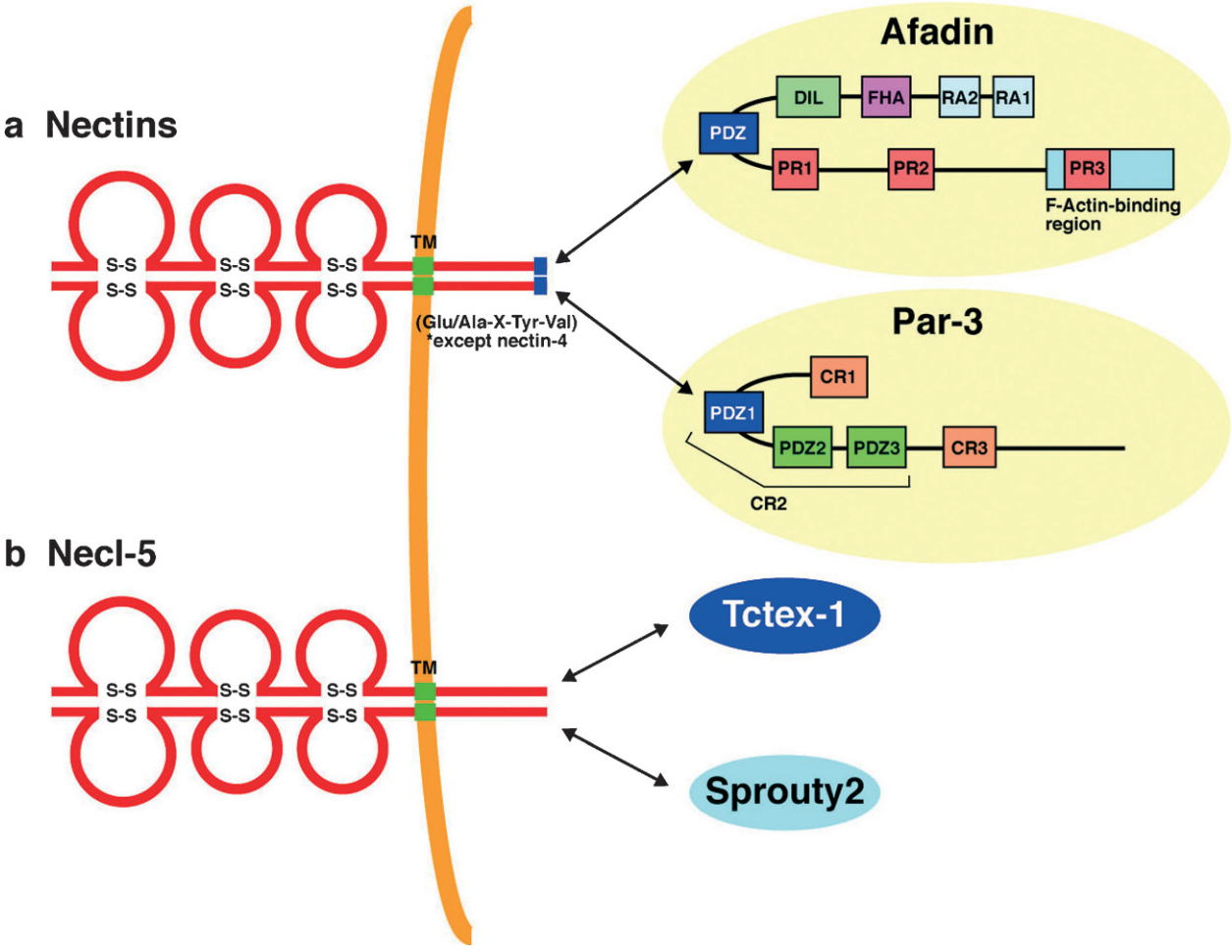

Figure 1. Molecular structures of nectins and Necl-5, and their interactions with peripheral membrane proteins. Nectins and Necl-5 form *cis*-dimers. Nectins and Necls consist of three extracellular Ig-like domains, a single transmembrane (TM) region, and a cytoplasmic tail. (*a*) Interactions of nectins with afadin and Par-3. Nectin-1, nectin-2, and nectin-3 possess a conserved motif with four amino acids (Glu/Ala-X-Tyr-Val) for interaction with afadin, while nectin-4 binds afadin through another C-terminal motif. Afadin has two Ras-association (RA) domains, a forkhead-associated (FHA) domain, a DIL domain, a PDZ domain, three proline-rich (PR) domains, and an F-actin-binding domain. Nectins directly bind the PDZ domain of afadin. Par-3 has three conserved regions (CR): CR1, CR2, and CR3. CR2 contains three PDZ domains. Nectin-1 and nectin-3 directly bind the first PDZ domain (PDZ1) of Par-3. (*b*) Interactions of Necl-5 with Tctex-1 and Sprouty2. Unlike nectins, Necl-5 lacks the C-terminal PDZ domain-binding motif. The cytoplasmic domain of Necl-5 interacts with Tctex-1, a light-chain subunit of cytoplasmic dyneins, and Sprouty2, a negative regulator of growth factor-induced signals inhibiting the activation of Ras.

nectins with afadin play an important role in the interaction of the nectin-afadin system with the cadherin-catenin system, however, the interactions of nectins with afadin alone are not sufficient for the formation of AJs [20, 21]. A cell polarity protein Par-3, which directly associates with nectins [22], promotes the interactions of nectins with afadin, and both Par-3 and afadin cooperatively regulate the formation of AJs [20]. In addition, three putative connector units have been implicated so far in the interactions between nectins and E-cadherin: a ponsin-vinculin unit, an ADIP-α-actinin unit, and an LMO7-α-actinin unit [6–12]. Ponsin apparently interacts with cadherin through vinculin, which connects α-catenin to F-actin, while ADIP and LMO7 are likely to associate with cadherin through α-actinin, which binds α-catenin. However, because ponsin does not form a ternary complex with afadin and vinculin, probably due to the competitive interaction of ponsin with afadin or vinculin [23], ponsin is unlikely to play a major role in the recruitment of the cadherin-catenin system to the nectin-based cell-cell adhesion sites. Ponsin might regulate the linkage between afadin and vinculin to promote the connection between nectin and cadherin. In addition, since the binding region of α-actinin with α-catenin overlaps with its binding region with ADIP and LMO7, it is unclear whether ADIP or LMO7 form a ternary complex with α-actinin and α-catenin. LMO7 does not appear to function as a molecule that recruits the cadherin-catenin system to the nectin-

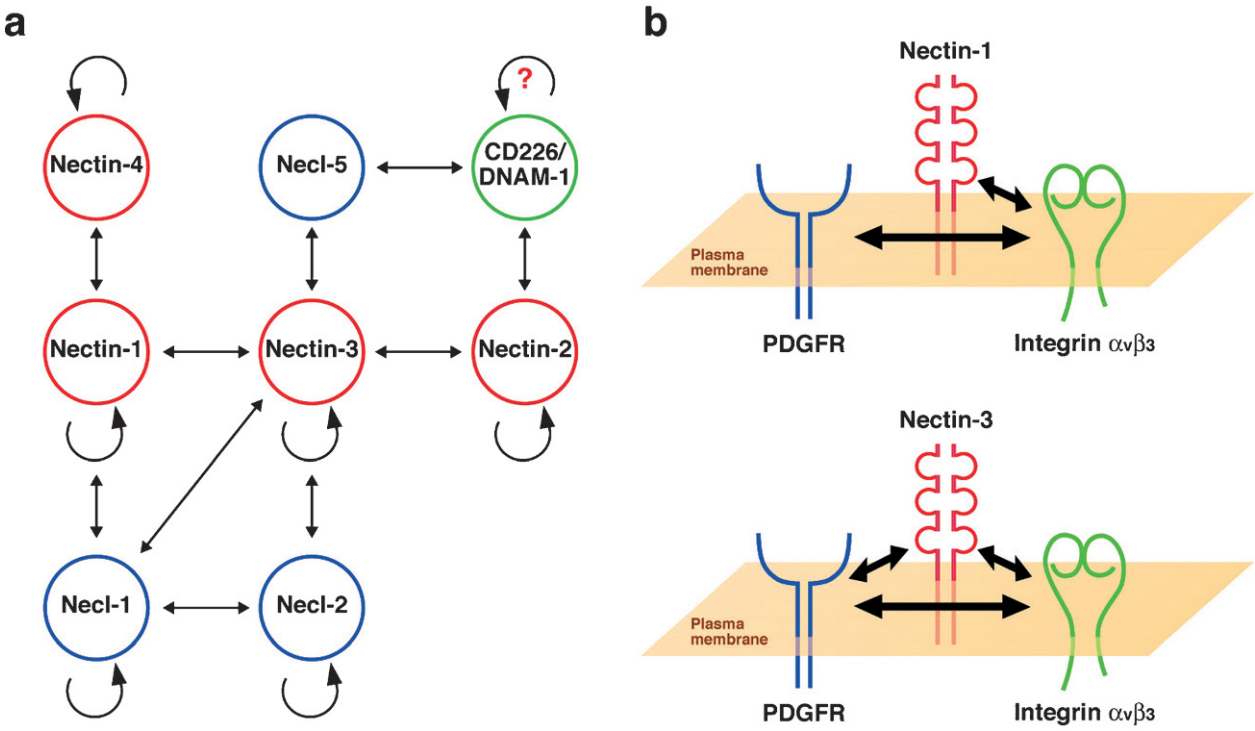

Figure 2. Interactions of nectins with other transmembrane proteins. (*a*) *Trans*-interactions among nectins, Necls, and CD226/DNAM-1. Only known homophilic (looped arrows) and heterophilic (bidirectional arrows) interactions are indicated. (*b*) *Cis*-interactions of nectins with integrin $\alpha_v\beta_3$ and PDGFR. Nectin-1 and nectin-3 individually *cis*-interact with integrin $\alpha_v\beta_3$ and form a binary complex. Nectin-3 but not nectin-1 *cis*-interacts with PDGFR and forms a binary complex. Integrin $\alpha_v\beta_3$ and PDGFR form a binary complex as well.

afadin system; it may, rather, stabilize both systems at the cell-cell adhesion sites by connecting them, because LMO7 is assembled at AJs after the nectin-induced formation of cadherin-based AJs has been established at the cell-cell adhesion sites. Further studies are needed to explain how these connector units organize the nectin-afadin and cadherin-catenin systems.

On the other hand, nectin-based cell-cell adhesion recruits intracellular signaling molecules to the adhesion sites and modulates their activities, allowing the reorganization of the actin cytoskeleton and the integrity of the intercellular junctions required for the establishment of cell-cell adhesion. The formation of nectin-based cell-cell adhesion, for example, induces the activation of Rap1, Cdc42, and Rac small G-proteins through activation of c-Src [24–26]. Activated Rap1 binds to afadin, which strengthens the binding of $p120^{ctn}$ to non-*trans*-interacting E-cadherin [27]. This association of the Rap1-afadin complex with the $p120^{ctn}$-E-cadherin complex inhibits endocytosis of non-*trans*-interacting E-cadherin and thereby enhances the accumulation of non-*trans*-interacting E-cadherin at the nectin-based cell-cell adhesion sites and cell-cell adhesion activity of E-cadherin, eventually forming AJs at the nectin-based cell-cell adhesion sites [21, 27]. Activated Cdc42 and Rac induce the reorganization of the actin cytoskeleton through an F-actin-cross-linking protein IQGAP1 [28], which then recruits non-*trans*-interacting E-cadherin to the nectin-based cell-cell adhesion sites [6–11]. In addition, activated Cdc42 induces filopodia formation and increases cell-cell contacts between apposing cells, whereas activated Rac promotes lamellipodia formation, thereby closing the gaps between cell-cell contact sites [6–11]. Collectively, nectin-based cell-cell adhesion regulates the reorganization of the actin cytoskeleton by two mechanisms: one mechanism is the recruitment of F-actin-binding proteins associated with nectins and/or E-cadherin, such as afadin, α-catenin, α-actinin, and vinculin; the other is the activation of Rap1-, Cdc42-, and Rac-dependent signaling pathways. Thus, the nectin-induced actin cytoskeletal reorganization results from exerting its function in cooperation with a wide variety of proteins, accelerating AJ formation and increasing cell-cell adhesion activity at AJs.

In addition, nectins indirectly associate with a Ca^{2+}- and phospholipid-binding protein annexin II and with IQGAP1, which link cell membranes to the actin cytoskeleton [29–31]. In annexin II-knockdown MDCK cells, the assembly of E-cadherin and its associated proteins is not observed at the nectin-based cell-cell adhesion sites and the formation of AJs is

impaired in the Ca^{2+} switch experiment [29]. Annexin II cooperates with other actin-binding proteins such as IQGAP1 and α-catenin during the formation of AJs [29]. Thus, annexin II is essentially involved in the formation of AJs.

Implication of *cis*-interactions of nectins with integrin $\alpha_v\beta_3$ through the extracellular regions in the formation of AJs

Integrin has at least two forms, an inactive low-affinity form with a weaker adhesion activity for extracellular matrix (ECM) and an active high-affinity form with a stronger adhesion activity for ECM [32]. Nectins are able to physically associate with both forms of integrin $\alpha_v\beta_3$. It has been reported that there is cross-talk between cell-cell and cell-matrix junctions [33, 34]. Integrin-mediated cell-matrix junctions positively or negatively regulate the formation and integrity of cell-cell junctions through the integrin-initiated outside-in signaling pathway, including protein kinase C (PKC), focal adhesion kinase (FAK), and c-Src [35, 36]. Nectins initially associate with the high-affinity form of integrin $\alpha_V\beta_3$ at the primordial nectin-based cell-cell adhesion sites during AJ formation. As AJ formation is established, the high-affinity form of integrin $\alpha_v\beta_3$ is gradually converted to the low-affinity form, which still associates with nectins at AJs [19, 37]. Nectin-mediated signals involve the activation of integrin $\alpha_v\beta_3$ as well as the integrin-initiated PKC-FAK-c-Src signaling pathway, which is necessary for the nectin-mediated signals for AJ formation [19, 37]. Indeed, the interactions of nectins with integrin $\alpha_v\beta_3$ are involved in the nectin-mediated activation of Cdc42 and Rac, which locate downstream of c-Src and are involved in the recruitment of E-cadherin to the nectin-based cell-cell adhesion sites to form AJs. Hence, the interactions of nectins and integrin $\alpha_v\beta_3$ play a pivotal role in cross-talk between cell-matrix and cell-cell junctions and in the formation of AJs. Activation of PKC is also required for the recruitment of TJ transmembrane proteins to the apical side of AJs [38–40].

Implication of interactions of nectins with cadherins through the cytoplasmic tails in the formation of puncta adherentia junctions

When neurons extend axons to dendrites to create interneuronal connections during the development of neural networks, axons selectively identify their appropriate partners. For example, axons in hippocampal pyramidal neurons bind dendrites to establish a stable synapse, whereas dendrites do not form stable contacts with dendrites. There are at least two types of interneuronal junctions between axon terminals and their targets at synapses: synaptic and puncta adherentia junctions [41]. Synaptic junctions act as sites for neurotransmission, consisting of active zones where Ca^{2+} channels localize and synaptic vesicles are docked and fused and postsynaptic densities where neurotransmitter receptors localize. Puncta adherentia junctions serve as mechanical adhesion sites with symmetrical paramembranous dense materials and no association with synaptic vesicles or postsynaptic densities, and appear to be ultrastructurally similar to adherens junctions of epithelial cells. In cultured rat hippocampal neurons, axons express nectin-1 and nectin-3, while dendrites express nectin-3 [42, 43]. Since the binding affinity of heterophilic *trans*-interaction between nectin-1 and nectin-3 is stronger than that of either homophilic *trans*-interaction [2], the heterophilic *trans*-interaction between nectin-1 on axons and nectin-3 on dendrites predominantly occurs when axons and dendrites create initial contacts. In cultured neurons, abnormal localization of nectin-1 to dendrites besides axons by ectopic overexpression of nectin-1 results in the formation of atypical dendro-dendritic and excessive axodendritic interactions [42], indicating that the controlled *trans*-interaction of nectin-1 on axons and nectin-3 on dendrites is critical for the well-ordered interaction between axons and dendrites. Nectin-based initial contacts recruit specific axonal and dendritic protein components including N-cadherin to this initial contact site, forming a functional synapse [42]. Consistently, despite the symmetrical localization of N-cadherin, nectin-1 and nectin-3 are localized asymmetrically on presynaptic and postsynaptic sides, respectively, of the plasma membranes of puncta adherentia junctions between the mossy fiber terminals and the pyramidal cell dendrites in the CA3 area of the hippocampus, and both nectins and N-cadherin are involved in the formation of puncta adherentia junctions [44]. Indeed, nectin-1-knockout mice as well as nectin-3-knockout mice show a decrease in puncta adherentia junctions at the synapses in the CA3 area of the hippocampus [45]. In summary, nectins play a role in the formation of puncta adherentia junctions in cooperation with N-cadherin.

Implication of nectin-based cell-cell adhesion in morphogenesis: lessons from knockout mice

Nectin-knockout mice are viable presumably owing to the functional redundancy within nectins and/or compensation by other CAMs for the absence of nectins. No compensatory up-regulation is observed in nectin knockout mice. Nevertheless, nectin-1 knockout mice and nectin-3 knockout mice show a developmental defect of the vitreous body [46], besides a decreased formation of puncta adherentia junctions at the synapses in the CA3 area of the hippocampus [45].

This phenotype, called microphthalmia, is characterized as malfunction of the ciliary body associated with a separation of the apex-apex adhesion between the pigment and non-pigment epithelia of the ciliary body. In addition, nectin-2 knockout mice and nectin-3 knockout mice show male-specific infertility due to differentiation abnormalities in the later stages of spermatogenesis, resulting from the structural disturbance of Sertoli cell-spermatid junctions by mislocalization of nectin-3 and nectin-2, respectively [47–49]. These observations indicate the essential role of the heterophilic interactions between nectin-2 on Sertoli cell and nectin-3 on spermatids. Hence, evidence gained from *in vivo* studies utilizing knockout mice shows that the heterophilic interactions between these nectins are particularly important in the integrity of these cell-cell adhesions.

Regulation of cell polarity

Nectins regulate not only AJ but also TJ formation; however, because the molecular mechanisms involved in the formation of TJs are apparently quite complicated, the role of nectins in TJ formation has not been completely elucidated. Nectins recruit TJ constituents such as JAMs, claudins, and occludin to the apical side of the nectin-based cell-cell adhesion sites, in cooperation with afadin, cell polarity proteins, cadherin, annexin II, IQGAP1, and ZO proteins [6–12]. Direct binding of nectin-1 and nectin-3 to the PDZ domain of Par-3 through the C-terminal four amino acids (Fig. 1a) is implicated in the establishment of epithelial cell polarization [22]. Par-3 forms a complex with other cell polarity proteins Par-6 and atypical PKC (aPKC) [50, 51]. In addition, actin-binding proteins such as annexin II and ZO proteins are reported to be critically involved in the formation of TJs. [29, 52, 53]. Thus, by forming large complexes at the cell membrane, nectins can function as organizers of membrane domains and membrane recruitment platforms for diverse proteins with which they interact.

Implication of nectin interactions with TJ proteins through the cytoplasmic tails in the formation of TJs

In polarized epithelial cells, TJs are formed at the apical side of AJs, appear to serve as a barrier preventing solutes and water from passing across the intercellular gaps and function as a fence between apical and basolateral plasma membranes. So far, several kinds of TJ transmembrane proteins have been identified. Among them, the major constituents of TJs, such as JAMs, claudins, and occludin, are Ca^{2+}-independent CAMs and have been most intensively characterized [14–16]. During or after AJ formation, nectins initially recruit JAMs to the adhesion sites, followed by the recruitment of claudins and occludin to the apical side of AJs in cooperation with E-cadherin, resulting in the formation of TJs [6–11]. Nectin-1 and nectin-3, but not nectin-2, directly interact with Par-3 through the C-terminal four amino acids of the cytoplasmic tail [22]. It is conceivable that the Par-3-Par-6-aPKC complex plays a central role in the formation of TJs [50, 51]. The concomitant activation of Cdc42 in response to nectin-mediated cell-cell adhesion results in the activation of the complex-associated aPKC activity through the binding of activated Cdc42 to Par-6. In addition to the formation of TJs, the Par-3-Par-6-aPKC complex is necessary for the formation of AJs [20, 54, 55]. This complex seems to be required for the associations of nectins with afadin, but it is dispensable for the formation of nectin-based cell-cell adhesion [20].

On the other hand, it is also conceivable that the associations of nectins with actin-binding proteins such as annexin II and ZO proteins are also involved in the formation of TJs [29, 52, 53]. In annexin II knockdown MDCK cells, although E-cadherin-based cell-cell adhesion is not formed, TJs and nectin-based cell-cell adhesion are formed. In other words, TJs can be formed in annexin II knockdown MDCK cells even in the absence of E-cadherin-based AJs, suggesting an inhibitory role of annexin II for TJ formation [29]. By binding to afadin, nectins indirectly associate with ZO proteins, which interact with JAMs, claudins, and occludin, and link these TJ transmembrane proteins to the actin cytoskeleton [6–12]. Taken together, nectins recruit TJ constituents through the associations with afadin and ZO proteins.

Regulation of cell survival

Once cells become confluent and intercellular junctions are established, cells terminate movement and proliferation and tend to maintain cellular homeostasis for survival [56, 57]. Nectin-based cell-cell adhesion plays an important role in PDGF-induced cell survival by preventing apoptosis through the activation of the phosphatidylinositol 3-kinase (PI3K)-Akt signaling pathway [58, 59, unpublished data].

Implication of the interaction of nectin-3 with PDGFR in the regulation of apoptosis

At cell-cell adhesion sites in NIH3T3 cells, nectin-3 interacts with PDGFR through the extracellular regions (Fig. 2b) (unpublished data). Serum depletion or Fas ligand can induce apoptosis in NIH3T3 cells and knockdown of nectin-3 or afadin in NIH3T3 cells

increases apoptotic cell numbers (unpublished data). PDGF can inhibit apoptosis in control NIH3T3 cells, whereas PDGF fails to prevent apoptosis in afadin-knockdown NIH3T3 cells, indicating the critical role of afadin in the anti-apoptotic effect of PDGF (unpublished data). In line with this, knockdown of nectin-3 or afadin results in the inhibition of the PDGF-induced activation of the PI3K-Akt signaling pathway, demonstrating the critical role of the nectin-afadin system for the PDGF-induced survival signals (unpublished data). The linkage of nectin-3 with afadin is necessary for the activation of the PI3K-Akt signaling pathway, because the transfection of the nectin-3 mutant that is unable to bind afadin in NIH3T3 cells fails to mediate the PDGF-induced phosphorylation of Akt (unpublished data). Indeed, embryoid bodies derived from afadin-null embryonic stem (ES) cells display an enormous number of apoptotic cells in their cavity in comparison with those from wild-type ES cells, indicating the anti-apoptotic effect of afadin (unpublished data). Thus, the nectin-afadin system regulates cell survival in cooperation with PDGFR.

Regulation of cell movement and proliferation

Nectins can *trans*-interact not only with nectins, but also with Necls [6–12]. Necls comprise a family of five members (Necl-1 to Necl-5), and unlike nectins, they do not bind afadin. In addition to the formation of homophilic dimer complexes, Necl-1, Necl-2, and Necl-5 can heterophilically *trans*-interact with other Necls and nectins. Nectin-1 binds Necl-1 while nectin-3 associates with Necl-1, Necl-2, and Necl-5 (Fig. 2a). The functional importance of interactions of nectins with Necls remains largely undetermined; however, studies to explore the role of the interaction of nectin-3 with Necl-5 have provided new insights into the molecular processes that regulate contact inhibition of cell movement and proliferation.

Implication of the interaction of nectin-3 with Necl-5 in the regulation of cell movement and proliferation

Cells in culture continuously move and proliferate until they become confluent. Once cells become confluent and form intercellular junctions, cells terminate both movement and proliferation [56, 57]. This phenomenon has been known for a long time as contact inhibition of cell movement and proliferation. However, the molecular mechanisms involved in contact inhibition of cell movement and proliferation have not yet been fully clarified. Presumably, cell-cell contact-induced *trans*-interaction of nectin-3 with Necl-5 (also termed as PVR/CD155/Tage4) and subsequent down-regulation of Necl-5 is one of the mechanisms involved in contact inhibition of cell movement and proliferation [60]. In moving NIH3T3 cells (in which Necl-5 does not *trans*-interact with nectin-3), Necl-5 localizes at the leading edges of the cells, where integrin $\alpha_v\beta_3$ and PDGFR colocalize [61, 62]. Necl-5, integrin $\alpha_v\beta_3$, and PDGFR cooperate with one another and promote signals, including Rac and Ras signaling [62, 63]. When two moving cells meet, Necl-5 heterophilically *trans*-interacts with nectin-3 on the adjacent cell surface to initiate the formation of intercellular junctions. The *trans*-interaction between Necl-5 and nectin-3 induces the activation of Cdc42 and Rac, leading to the reorganization of the actin cytoskeleton and to increasing cell-cell adhesion sites [64]. The *trans*-interaction of Necl-5 with nectin-3 is transient, and Necl-5 is then down-regulated from the cell surface by clathrin-dependent endocytosis [60], which leads to inhibition of cell movement and proliferation. On the other hand, nectin-3 dissociated from Necl-5 is retained on the cell surface and subsequently *trans*-interacts with nectin-1 [6–11]. The *trans*-interaction between nectin-1 and nectin-3 induces E-cadherin recruitment to the nectin-based cell-cell adhesion sites, eventually establishing AJs. Hence, nectin-3 regulates cell movement and proliferation by interacting with Necl-5.

Microtubules (MTs) are implicated in directional cell movement as their networks are reoriented and directed toward leading edges during directional cell movement [65]. The reorientation of the MT networks is regulated by the activation of Cdc42, Rac, and cell polarity proteins, such as Par-3, aPKC, and Par-6 [65, 66], and also depends on the processes of searching for a membrane cue and capturing of the plus ends of MTs at leading edges [67]. The activation of Rac at leading edges leads growing/pioneering MTs during the searching process [68]. Many proteins localize as plus-end-binding proteins (+TIPs): these include cytoplasmic dynein/dynactin, cytoplasmic linker proteins (CLIPs), and EB1 [67]. When the plus ends of MTs find a membrane cue, they are captured and stabilized at the rear sites of the leading edges. The capturing and stabilization are regulated by the complex formation of CLIP-associating proteins (CLASPs), ELKs, and LL5β, which are also +TIPs [69]. Necl-5 functions as a membrane cue for attracting growing MTs at the leading edges of moving NIH3T3 cells in the processes of searching and capturing of the plus ends of MTs (unpublished data). Necl-5 directly interacts with Tctex-1 (Fig. 1b), a light-chain subunit of cytoplasmic dynein that binds to growing plus ends of MTs and recruits MTs to the leading edges. The interaction of Necl-5 with the dynein/dynactin complex regulates the

searching of MTs at the leading edges. Thus, the interaction of Necl-5 and Tctex-1 plays a key role in the searching process of growing MTs in moving NIH3T3 cells. It has consistently been reported that the cytoplasmic domain of human Necl-5/PVR/CD155 directly interacts with Tctex-1 in neurons [70, 71]. Since the dynein motor complex creates the major driving force for minus end-directed transport along MTs, the direct interaction between Necl-5/PVR/CD155 and Tctex-1 appears to be essential for the axonal retrograde transport of poliovirus-containing vesicles.

Sprouty (Spry) is a novel family of negative regulators of growth factor-induced signals [72]. Spry is directly tyrosine-phosphorylated by c-Src and activated in response to various growth factors [72]. Active Spry inhibits the growth factor-induced activation of Ras, thereby inhibiting the activation of the Raf-mitogen-activated protein kinase kinase (MEK)-extracellular signal-regulated kinase (ERK) signaling pathway, but affects neither phosphorylation of growth factor receptor nor activation of c-Src and PI3K [73]. Necl-5 physically and functionally associates with Spry2 (Fig. 1b), and the association of Necl-5 with Spry2 is involved in the regulation of the PDGF-induced Ras signaling and proliferation [74]. When cells do not contact other cells, Necl-5 interacts with Spry2, thereby preventing it from inhibiting the PDGF-induced activation of Ras. On the other hand, when cells contact other cells, Necl-5 is down-regulated by interacting with nectin-3, thereby releasing Spry2 from Necl-5. Released active Spry2 subsequently inhibits the PDGF-induced activation of Ras. Collectively, it is likely that suppression of the PDGF-induced Ras signaling by Necl-5-regulated Spry2 is involved in contact inhibition of cell proliferation.

Regulation of other cellular functions

Nectin-2 *trans*-interacts with CD226/DNAX accessory molecule-1 (DNAM-1), an Ig-like CAM broadly expressed on the surface of peripheral leukocytes including T cells, natural killer (NK) cells, monocytes, and a subset of B cells as well as platelets (Fig. 2a) [75–77]. So far, several lines of evidence have indicated that the interaction of nectin-2 with DNAM-1 contributes to T cell- and NK-mediated cytotoxity, immune response, and tumor immunity [78, 79]. A recent report demonstrates that human mast cells and eosinophils express nectin-2 and CD226/DNAM-1 and suggests that the interaction between nectin-2 and CD226/DNAM-1 can allow allergic reactions through costimulatory receptor/ligand interactions, because blocking nectin-2 expressed on eosinophils by neutralizing antibodies normalizes the hyperactivation in mast cells resulting from IgE-dependent activation of mast cells cocultured with eosinophils [80]. This finding implies that blocking this interaction might potentially be a novel therapeutic strategy for allergic diseases.

Nectin-1 regulates the expression of loricrin, a major constituent of the cornified cell envelope of the epidermis, in keratinocytes, demonstrating a novel role of nectin-1 in gene expression [81]. Nectin-1 induces the activation of the Rap1-ERK signaling pathway, which mediates the up-regulation of loricrin, while expression of loricrin is down-regulated in nectin-1-null keratinocytes. Thus, nectin-1 plays a critical role for loricrin expression in the skin.

Conclusions and future directions

In this review, we summarized recent progress in our understanding of the implication of interactions of nectins with transmembrane and peripheral membrane proteins to exert pleiotropic functions. Evidence obtained from *in vitro* experiments and *in vivo* studies utilizing animal models including knockout mice has demonstrated that nectins play a variety of cellular functions, such as cell adhesion, polarization, survival, movement, and proliferation (Fig. 3). However, *in vivo* evidence concerning the associations of nectins with integrin $\alpha_v\beta_3$ and PDGFR is still missing. Further studies are needed to clarify whether nectins can interact with other integrins and/or growth factor receptors, and if so, the roles of these interactions should be elucidated.

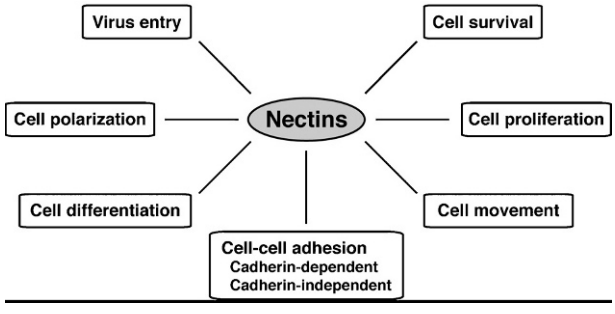

Figure 3. Pleiotropic functions of nectins. Nectins regulate a variety of cellular functions, including virus entry, cadherin-dependent and –independent cell-cell adhesion, polarization, survival, differentiation, movement, and proliferation.

Moreover, the data concerning implications of nectins in human disorders are scanty. Nectins are known to serve as viral entry receptors for alpha-herpes viruses including herpes simplex viruses (HSV-1 and HSV-2),

pseudorabies virus, and bovine herpesvirus type 1 [82–84]. Mutations of the *nectin-1* gene are observed in Zlotogora-Ogür syndrome and Margarita Island ectodermal dysplasia, which are characterized by an unusual face, dental anomalies, hypotrichosis, palmoplantar hyperkeratosis and onychodysplasia, syndactyly, cleft lip/palate, and in some cases, mental retardation [85, 86]. In addition, a potential role of nectin-4 as a novel histological and serological tumor-associated marker for breast cancer has been proposed [87, 88]. Nectin-4 is not expressed in normal breast epithelial cells, but highly expressed both in breast cancer and tumor cell lines. Since it was reported that other nectins, including nectin-1, are overexpressed in squamous cell carcinomas [89], nectins might be potentially utilized as a serum marker for patients with cancer. The diagnostic and prognostic significance of nectin expression in leukemia has also been reported [90]. Analysis of nectin-1 and nectin-2 expression in bone marrow cells from patients with acute myeloid leukemia yields important information about diagnostic criteria as well as prognosis. Studies to explore the pathological roles of nectins, particularly in the fields of oncology, neuroscience, and cardiovascular diseases, using epidemiologic, genetic, genomic, and proteomic approaches, will therefore provide further information to identify whether nectins could be a diagnostic marker and/or a potential therapeutic target for human diseases, and if so, may guide the development of new strategies for diagnosis and therapy.

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