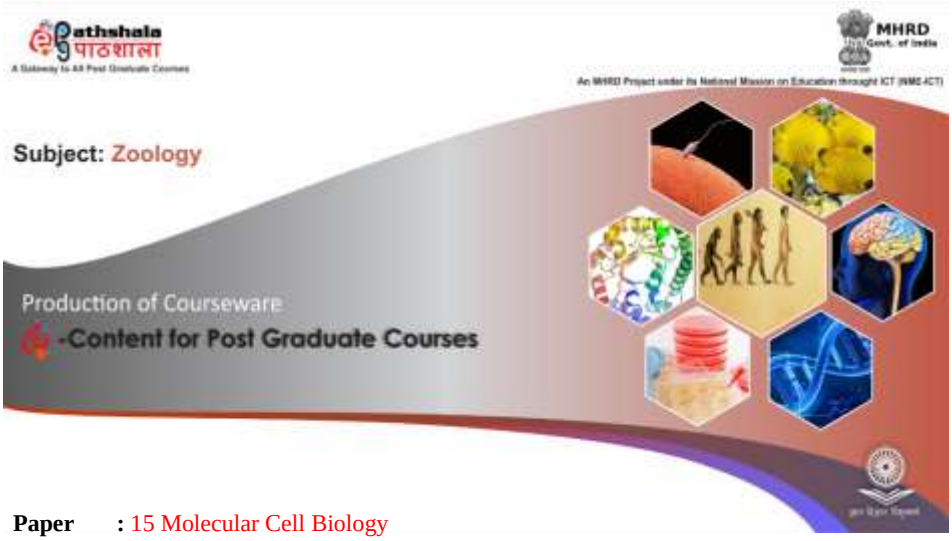


**Paper** : 15 Molecular Cell Biology
**Module** : 15 Cell-Cell Adhesion and Communication:
Cell-Cell Adhesion & Cell-ECM interactions

## Development Team


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| Description of Module | |
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| **Subject Name** | ZOOLOGY |
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| **Keywords** | Tight junctions, integrins, desmosomes, hemi-desmosomes, LICAMs. |

## Contents

## 1. Learning Outcomes

After studying this module, you shall be able to

- appreciate the importance of cell-cell interactions in the important cellular processes of multicellular organisms
- understand different types of cell adhesion molecules
- describe various kinds of possible cell-cell interactions
- understand the role of adhesion molecules in different human diseases

## 2. Introduction

The evolution of multicellular organisms was not mere collection of cells, but it required the *ex nihilo* creation of a completely new system of cellular organization in which cells cooperate with each other to form a multicellular organism. This intricate system of multi-cellularity involves the adhesion of cells with each other as well as with the extracellular matrix, which together form the tissue architecture and help in proper functioning of the tissues and the organs. The cell-cell adhesion requires multiprotein complexes and the associated cells not just 'stick' together but they also communicate with each other by cellular junctions and chemical signaling. In this chapter, the concept of cell-cell adhesion will be discussed in detail, followed by brief description of cellular junctions, but one of the important cell junctions, called gap junction has been discussed in detail in the next chapter.

## 3. Cell-Cell Adhesion

The adhesive (or anchoring) junctions connect cells together into tissues hence, defining a functional role of the unit. Cytoskeletal network is held to the cell surface to sustain tissue integrity and to withstand mechanical stress.

All categories of cell-cell adhesive junctions function by two component system:

i. *Intracellular attachment proteins*: mediate attachment of cytoskeletal filaments on the inside of the plasma membrane to the junction.
ii. *Cadherins:* overhang from cell surface and inter connect cells to each other.

### *3.1. Anchoring junctions*

Anchoring junction complexes are membrane-spanning structures that tether the plasma membrane to the tension-bearing filaments of the cytoskeleton on the inside of the cell as well as to neighboring cells or the ECM. Apart from anchoring the cells, several signaling proteins are a part of the molecular complexes and hence cellular signaling pathways are also synchronized.

Based on the molecular components and function, the anchoring junctions are categorized into two groups:

i. Adherens junctions and desmosomes: anchor the cells to each other and transmembrane adhesion proteins belong to the *cadherin* family.

ii. Focal adhesions and hemidesmosomes: attach cells to the ECM while the transmembrane adhesion proteins are of the *integrin* family

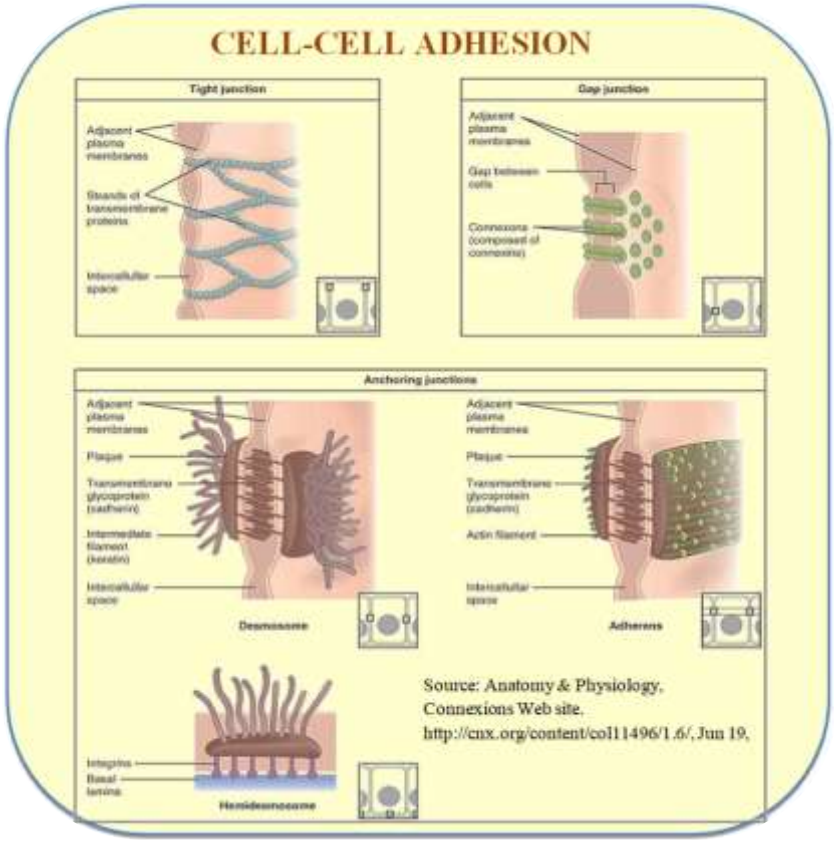



Fig 1. Types of Cell Junction

It is important to note here that while the cytoskeletal material binding the anchoring junctions is *actin* for adherens junctions and focal adhesion; *intermediate filaments* are involved for desmosomes and hemidesmosomes.

### *3.1.1. Adherens junctions*

- These are identified by actin as the principal cytoskeletal material connecting the calcium binding cadherin-mediated adhesive junctions.
- The space between the adjacent membranes is about 20–25 nm.
- Especially prominent in epithelial cells wherein these junctions form a continuous zone of attachment called the *zona adherens, encircling* the cell near the apical end of the lateral membrane thus, establishing the apicobasal polarity. They function as mechanosensors and serve as a nexus for signaling affecting important cell decisions, such as survival and differentiation
- For non-epithelial cells, the adherens junctions are represented as small punctate or streak like attachments.
- The β-catenin, a prominent cytosolic protein binds to the cytosolic tail of the cadherin and performs numerous roles in the cell notably in the Wnt signaling pathway important in cancer. The α-catenin is also recruited for the attachment of actin to the junction.
- The third major core component of adherens junctions is p120catenin (p120ctn). Like the α-catenin, p120ctn also binds to the cytoplasmic tail of cadherins near the plasma membrane. It helps in regulating both the stability of cadherin at the surface, as well as the activity of Rho, an actin regulator.

### *3.1.2. Desmosomes*

- Desmosomes are localized button-like points of strong union between adjacent cells in a tissue abundantly found in skin, heart muscle and the neck of the uterus.
- Apart from providing structural integrity, desmosomes also enable cell to resist stress.

- The plasma membranes of the two neighboring cells run parallel to each other and separated by an extracellular space of about 25–35 nm called the *desmosome core*.
- Adhesion proteins viz., desmosomal cadherins, desmocollin and desmoglein are present in the core and linked by their cytosolic tail to cytoskeleton. The plakoglobin binds to desmocollin and the intracellular anchor protein i.e., desmoplakin which in turn is connected to tonofilaments, the intermediate filaments comprising of vimentin, desmin, or keratin.
- An assemblage of these linker proteins and tonofilaments form a *plaqu*e underneath the plasma membrane of partnering cells.

### *3.1.3. Hemidesmosomes*

- Hemidesmosomes are the junctions between epithelial cells and underlying connective tissue (ECM). These occur on the inner basal surface of keratinocytes in the epidermis of skin.
- While desmosomes link two cells together, hemidesmosomes (HD) attach one cell to the extracellular matrix.
- Desmogleins are absent instead integrins are used for attaching the cell to ECM.
- The keratin filaments are linked by different members of the plakin family (e.g., plectin) to integrins.
- The HD comprises two rivet-like plaques (the inner and outer plaques). Together with the anchoring fibrils and anchoring filaments, these are collectively termed the HD-stable adhesion complex or HD-anchoring filament complex. Together, the HD-anchoring filament complex forms a continuous structural link between the basal keratinocyte keratin intermediate filaments and the underlying basement membrane zone (BMZ) and dermal components.

### *3.1.4. Focal, fibrillar, and 3-D adhesions*

- Focal adhesions enable cells to anchor themselves to the ECM through transmembrane integrins that link intracellularly to actin filaments.
- This interaction is mediated by several intracellular anchor proteins such as talin, a-actinin, filamin, and vinculin.

### *3.2. Tight junctions*

- As the name suggests, these serve to seal the epithelial tissues tightly to form barriers between the internal cells of the body and the outside world. These are abundant in epithelium lining organs (ducts and cavities of glands of digestive tract, such as the liver and pancreas) or body cavity (urinary bladder). For instance, this enables the intestinal cells to prevent fluids from passing through the digestive tract from the internal fluids of the body.

- The components of tight junctions are arranged to form a continuous belt near the apical end, lying just above the adherens junction. These belts form an effective barrier restricting transport of molecule in and out of the cell.

- Each junction is made from interconnected network extending giving criss-cross ridge shaped appearance under freeze-fracture microscopy. Individual ridge consists of a continuous row of tightly packed transmembrane junctional proteins about 3-4 nm in diameter. These are further fused to eliminate any intercellular spaces. In addition to these close membrane appositions, scaffolding proteins at tight junctions recruit cytoskeletal proteins, such as F-actin, to tight junctions.

- An analogy of tight junctions can be made with ‘gates’ preventing movement of molecules through the lateral margin and with ‘fences’ blocking the lateral movement of lipids in the outer membrane and transmembrane proteins entirely.

- Three important transmembrane proteins of tight junctions include: occluding; immunoglobulin superfamily proteins called the *junctional adhesion molecules* (JAMs); and *claudins*.

- The structure of claudins consists of four membrane-spanning domains with the largest extracellular loop comprising of charged amino acids facilitating the passage of specific ions. Such loops of adjacent cells are fused to form ion-selective pores and transport occurs *paracellularly* i.e., ions move between the cells.

***3.3. Gap junctions.***

- These can explained as regions of cytoplasmic contact between plasma membranes of two cells with a gap of only 2–3 nm in between. It acts a molecular pipeline for the transport of ions and small molecules.
- Such cellular connections are significant as these allow adjacent cells to be in direct electrical and chemical communication with each other.
- The functional and molecular details pf gap junctions are discussed in detail in separate chapter of the module by the authors.

## 4. Components of cell adhesion

Cell adhesion is important in tissue formation as well as cell motility and it is mediated by *cell-adhesion molecules or CAMs* or adhesion receptors, extracellular matrix (ECM) proteins and cytoplasmic plaque or peripheral membrane proteins. CAMs were initially identified when antibodies against the cell surface molecules inhibited cell-cell adhesion under *in vitro* conditions. The transmembrane cell adhesion molecules are linked through the adaptor proteins in cytoplasmic plaques to the cytoskeleton in all the cell-adhesion junctions.

Principally, the adhesion molecules or receptors are transmembrane glycoproteins and consist of five major classes, namely: cadherins, immunoglobulin (Ig) superfamily, selectins, mucins and integrins. These either form stable cell-cell adhesions or are involved in cell-matrix adhesion and help in cell migration or cell-cell interactions.

## 5. Cadherins

One of the major CAMs consists of a large family of cell surface proteins called cadherins. Cadherin is a transmembrane proteins of ~700-750 amino acids in length and consists of extracellular (EC), transmembrane and a cytoplasmic domain (Fig. 1). The EC domain contains repeated sequences which act as $Ca^{2+}$ binding sites and hence the name “cadherin”. The cell adhesion is mediated though the EC domains or cadherin repeats of opposing cells. Cadherins have a long evolutionary timeline because even ancestral metazoan species like

placozoans and cnidarians express several distinct cadherins and the expansion of cadherins accords with the emergence of vertebrates with major diversification reflected in higher metazoans. (Gul et al., 2017, Hirano et al., 2003).There are more than 100 members in the cadherin family, which mediate $Ca^{2+}$ dependent cell adhesion in vertebrate as well as invertebrate tissues.

Cadherins are further divided as 6 subfamilies, namely: classical cadherins, desmosomal cadherins, protocadherins, Flamingo/Celsr, and Dachsous and Fat cadherins. *Classical cadherins* were the first to be identified with 5 cadherin repeats with critical function in adherens junctions. They have highly conserved cytoplasmic region with binding sites for proteins like p120 and β-catenin, which link cadherins with the cellular cytoskeleton. *Desmosomal cadherins* are specific to the desmosome, also have five cadherin repeats, but they are linked with intermediate filaments through plakoglobin/desmoplakin. The next four subfamilies play a distinctive role than the classical and desmosomalcadherins. *Protocadherins* have variable number of cadherin repeats (5-27) and cytoplasmic region. Members of this subfamily like CDH23, which is a component of the tip linker of stereocilia and PAPC helps in convergence and extension movements during *Xenopus* gastrulation. *Flamingo/Celsr* is a non-classical cadherin and regulates wing bristle patterning and ommatidial rotation in *Drosophila,* the development of a normal array of stereocilia and neurite patterning in mammals. *Dachsous* is a large cadherin molecule with 27cadherin repeats. It is named on the "dachsous" gene of *Drosophila, which* regulates morphogenesis of the thorax, wings and legs. The *Fat* cadherin is also a large cadherin with 34 cadherin repeats and exists in both invertebrates and vertebrates (Tanoue & Takeichi, 2005).

Based on the specific structural features, cadherins are also classified as type I, type II and type III or atypical cadherins. Type-I/II cadherins are found only in vertebrates and ascidians. Type I cadherins consist of a unique histidine-alanine-valine (HAV) motif in their amino acid sequence and a single tryptophan (W) residue before the first calcium binding site in the N-terminus which may help in cell-cell interactions mediated by cadherins (Fig. 2). Type II cadherins also possess this HAV motif but have two tryptophan (WW) residues. On the other hand, Type III or atypical cadherins possess only the calcium binding sites with no HAV or

W residues in their amino acid sequence. Type II cadherins like cadherin-5, also called vascular endothelial cadherin, is found on endothelial cells and is part of the adherens junction that maintains blood vessel integrity. Type III classic cadherins are found in both vertebrates and invertebrates (Oda et al., 2002; Tanabe et al., 2004), but are absent from mammals. They are attached to the plasma membrane by a glycosyl phosphatedylinositol (GPI) anchor and lack transmembrane and cytoplasmic domain. Members of this family like cadherin-13, which is also referred to as T- or H-cadherin, is expressed in multiple cell types in the mammary gland, including myoepithelial, epithelial and endothelial cells (Andrews et al., 2012). The T-cadherin localizes adiponectin, an adipocyte-secreted protein to the vascular endothelium during the revascularization response to chronic ischemia (Parker-Duffen et al., 2013).

The first three discovered Type I cadherins were named according to their presence in respective tissues, like E-cadherin is present on different types of epithelial cells, N-cadherin on nerve, muscle and lens cells and P-cadherin present on cells of placenta and epidermis. However, these three cadherins are the most common family members and are also present in other tissues, for example, large number of E-cadherin are present in brain tissues and N-cadherin in fibroblasts. Usually, there are 5 cadherin repeats in Type I cadherins but their number can vary, for instance, E-cadherin & N-cadherin in *Drosophila* have six and 15 EC domains respectively (Suzuki & Takeichi, 2008).

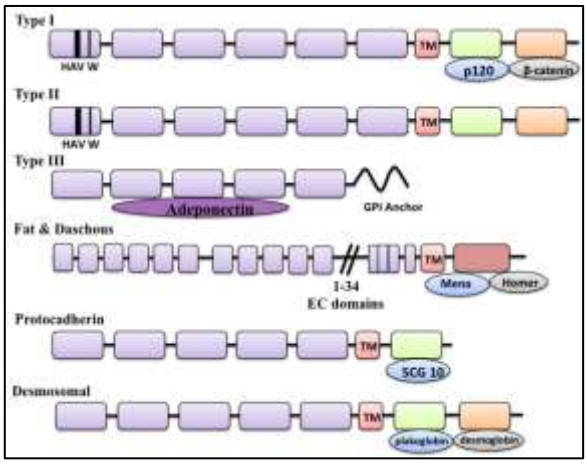
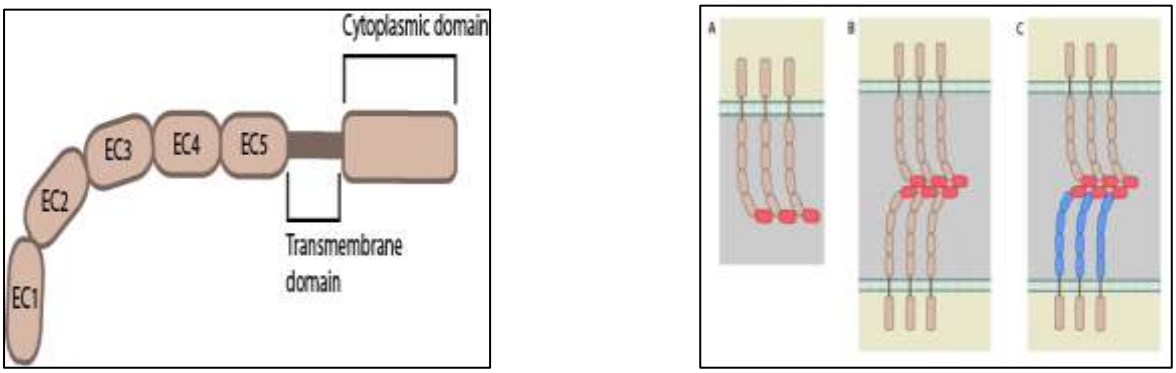


**Fig. 2:** Structure of different classes of cadherins with distinct characteristics in both the extracellular and cytoplasmic domains. The EC domains are depicted in purple boxes, TM: transmembrane in red boxes, cytoplasmic domains in green and orange boxes. Mena and Homer are intracellular anchor proteins with which Fat cadherins bind with cytoskeletal actin proteins; SCG10 is a microtubule-destabilizing protein.
**Source:** Author

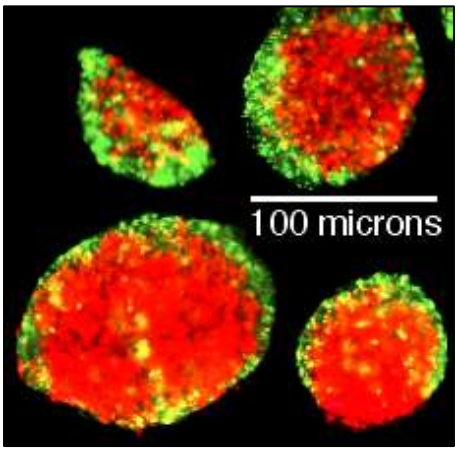
The binding of $Ca^{2+}$ is very crucial for the cadherin mediated cell adhesion because in the absence of $Ca^{2+}$, the binding of cadherins of two opposing cells is very weak. It is only after the binding of $Ca^{2+}$, the interaction becomes very stable and stiff because the $Ca^{2+}$ are positioned in between the cadherin repeats, thereby locking the repeats together into a stiff, rod like structure. Also, there is an additional level of rigidity, which is attained after the conformational changes induced from binding of individual cadherins of two cells together. It is observed that, if all the $Ca^{2+}$binding sites of cadherins are not occupied in case of low extracellular $Ca^{2+}$ concentration, then the extracellular part of cadherin protein becomes droopy and is finally degraded by proteolysis. (Gumbiner, 1996, Lodish et al., 2000, Alberts et al., 2002).

The cadherin mediated intercellular interactions either include *is interactions*, wherein, Type-I cadherins engage in lateral interactions on the same cell, or *trans interactions* which are commonly *homophillic interactions*, which involves binding with same type of cadherin on opposing cells, but *heterophillic interactions*, which consists of binding with different cadherin molecules on the neighboring cells (Fig. 3b) are also present. In fact, the specificity of cadherin mediated adhesion, (like N-cadherin interacts with N-cadherin, whereas E-cadherin interacts with E-cadherin), has provided a molecular basis for "*cell sorting*" seen in morphogenesis during embryonic development. This phenomenon was explained by Malcolm Steinberg through his *Differential Adhesion Hypothesis*, which explains that when cells share same cell adhesion molecules, then the homotypic interactions between these cells can maintain boundaries between groups of those cells with different CAMs. Also, the number and different types of adhesion molecules influence the strength of cell adhesion across the cell surfaces (Fig. 3c).




(a) (b)

**Fig. 3(a)** Structure of cadherin protein,
**(b)** Cadherin mediated cellular interactions: A: cis interaction, B: homophillic and C: heterophillic interaction. (**Source:**https://www.mechanobio.info/resources/proteins/cadherin/)




**(c):** Cell sorting, cells. LiveP19 embryonal carcinoma cells were stained with either DiI (red) or DiO (green). The red cells were genetically altered and express higher levels of E-cadherin than the green cells. Note the aggregation of green and red cells in separate boundaries with no intermixing of both cells.
**(Source:** https://en.wikipedia.org/wiki/Morphogenesis)

The cytoplasmic tail of cadherin interacts with the actin cytoskeleton mediated by two anchor proteins: α-catenin andβ-catenin and an intracellular protein-p120-catenin (Fig.4) to form the so-called *zonula adherens* or adherens junction.α-catenin binds to β-catenin, which further binds in a region of ~96 amino acids near the C-terminus of the cytoplasmic domain of cadherin, while p120-catenin attaches directly to the cytoplasmic tail at the membrane-proximal region. The binding of these proteins is crucial for the proper functioning and stability of cadherin. Cadherins also interact with other adaptor proteins like vinculin as well

as non-muscle myosins at the *zonula adherens* to form linkages between cell membrane and actin cytoskeleton. Thus, it is clear that cadherins regulate the dynamic actin cytoskeleton (Liang et al., 2015).

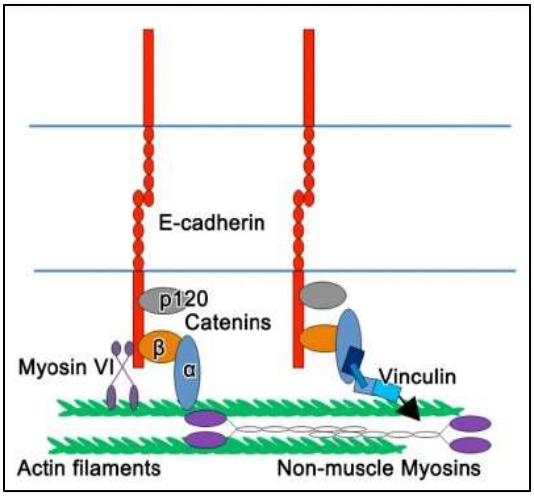



**Fig. 4:** Interaction of cadherin with cytoskeletal proteins.
**(Source:** Liang et al., 2015, https://www.dovepress.com/current-perspectives-on-cadherin-cytoskeleton-interactions-and-dynamic-peer-reviewed-fulltext-article-CHC)

### *5.1. Functions of Cadherins*

The role of cadherins in *tissue organization* was established from studies on *Xenopus* oocytes, wherein the expression of E-cadherin mRNA was inhibited using antisense deoxyoligonucleotide sand the resulting disaggregated blastulae showed loss of adhesion between blastomeres and obliteration of blastocoel (Heasman et al. 1994, Fig. 5). Type II cadherins are involved in different cellular functions like neural patterning, cell migration, axon guidance, synapse formation, and synapse function as revealed though gain and loss-of-function mutation analyses (Matsunaga et al., 2015).

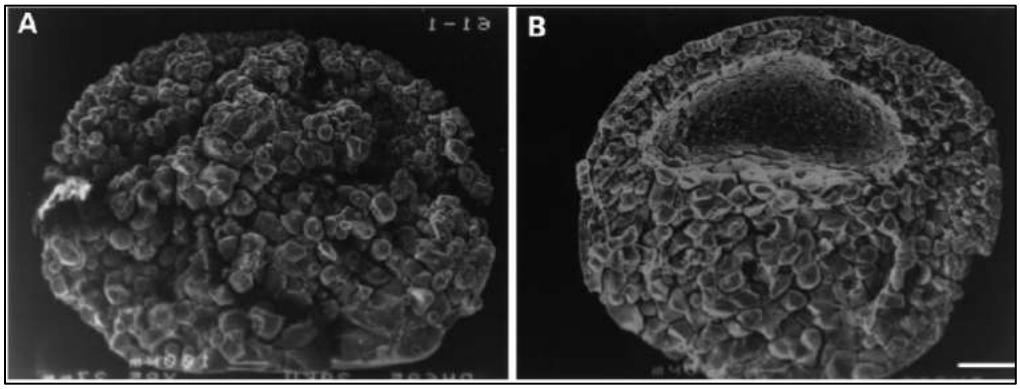



**Fig. 5:** Scanning electron micrographs of Xenopus blastulae derived from (A) antisense deoxyoligonucleotides & (B) control oocytes, Bar-0.17 mm (**Source:** Heasman et al. 1994).

Loss of function studies from mutations in N-cadherin genes in zebrafish suggested that N-cadherin helps in *rearrangement of neuroepithelial cells* wherein the neuroectodermalcells first converge at the midline followed by the cavitation of neural tube. Loss of N-cad results in blockage of neural tube formation in the midbrain-hindbrain region and several other neural defects (Lele et al., 2002). Grunwald et al first discovered N-cadherin in chick neural retina in 1982, followed by its identification in adherens junction by Volk and Geiger, 1984.

Further, N-cadherin *stabilizes synapses* in terms of regulating the spine head width which has been extensively studied in hippocampal neurons (Mendez et al. 2010, Arikkath, 2010). The dynamic nature of dendritic spines is exhibited by their active formation and elimination. During excitatory synaptic transmission, PSD-95, a postsynaptic marker is expressed and gets accumulated at the activated synapse (Fig. 6). Also, N-cadherin is recruited to the synapses.

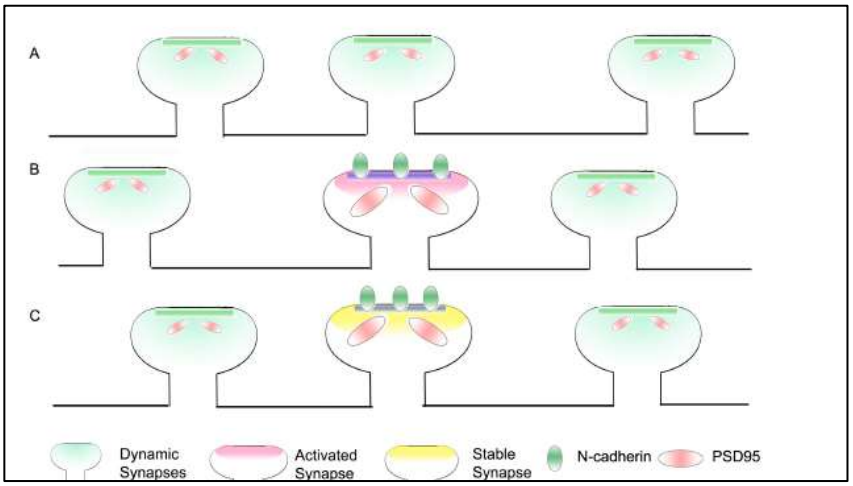


**Fig. 6:** Role of N-cadherin in promoting dendritic spine stability. (A) Spines are dynamic in nature with continuous and undergo formation and elimination, leading to spine turnover. These spines have relatively small postsynaptic densities and PSD-95 puncta. (B) Localization of N-cadherin to activated synapses is promoted bysynaptic activity. (C) N-cadherin stabilizes theactivated synapses and large postsynaptic densities and expresses large PSD-95 puncta.

In addition to their role in stabilizing synapses, cadherins like E-cadherin & N-cadherin play an important role in *cell migration*. Cell migration is a fundamental process in normal embryonic development, tissue repair or wound healing and disease progression like metastasis of tumor cells. Usually, E-cadherin molecules pack the cells tightly, but during cell migration, the epithelial cells undergo a process called as complete or partial EMT (epithelial to mesenchymal transition, Fig. 7), wherein, as the name suggests, the epithelial cells progressively lose their characteristics and acquire the features of mesenchymal cells with increased migratory and invasive properties. The epithelial characters which are lost include- a) an organized apical-basal polarity, b) lowered expression or down regulation of E-cadherins, integrins and other cell-surface proteins, thus destabilizing the cell-cell contacts, c) loss of junctional complexes like tight and adherent junctions, d) shift towards the transcription of mesenchymal genes and suppressing epithelial marker genes by different transcription factors and e) increased expression of cytoskeletal proteins. Thus, with an increased expression of cytoskeletal proteins, actin filaments with the adaptor proteins push the cell membrane in outward direction with a leading edge called filopodia, followed by formation of cell-matrix interactions and focal adhesion complexes. This is followed by contraction of cell by actomyosin activation, focal proteolysis and detachment of tail from the substratum and hence the cell moves forward (Ribeiro & Paredes, 2015, Derycke & Bracke, 2004).

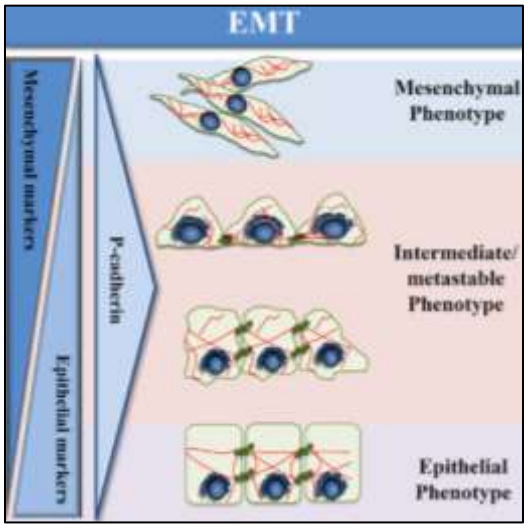


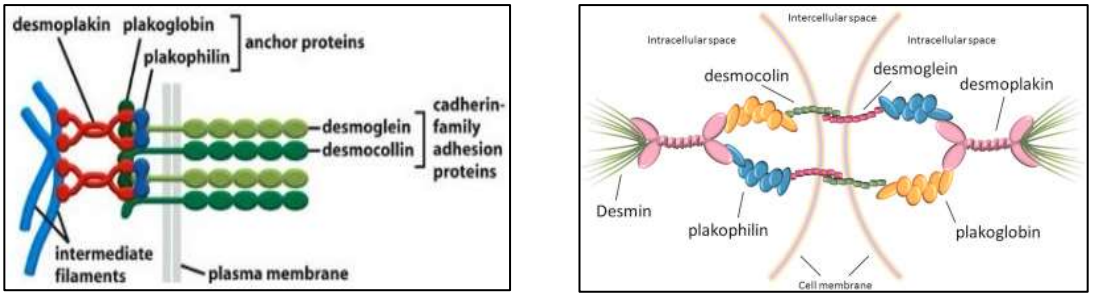

**Fig. 7:** Epithelial to mesenchymal transition (EMT)
**(Source:** Ribeiro & Paredes, 2015,http://journal.frontiersin.org/article/10.3389/fonc.2014.00371/full)

In case of *cancer progression*, the EMT characterizes up regulation of N-cadherin in metastatic tumors in addition to down regulation of E-cadherin. In contrast, in case of neural crest cells, there is loss of N-cadherin expression and gain in expression of cadherin-6B (expressed in neural folds) and cadherin-7 (in neural tube cells) (Shih &Yamada, 2015). Thus, depending on their specific role in embryonic development, cell differentiation and cancer cell invasion, the switching of E-cadherin to N-cadherin or vice versa can take place. However, in breast-cell carcinomas, P-cadherin is overexpressed which promotes increased tumor cell motility and invasiveness through degradation of ECM by matrix metalloproteases (MMPs) (Ribeiro & Paredes, 2015).

### *5.2. Desmosomes*

The multiprotein complex mediated by cadherins can also bind to intermediate filaments instead of actin filaments in a structure named as **desmosome** and consists of different anchor or adaptor proteins, namely plakoglobin (Pg), plakophillin (Pkps) and intracellular domains of cadherin proteins: desmogleins (Dsgs) and desmocollins (Dscs) (Fig. 8a). Dsgs and Dscs form heterodimers, providing an adhesive link between two neighboring cells in the transmembrane space, while in the outer dense plaque region of the cell, the cadherin proteins (Dsgs & Dscs) associate with Pg and Pkps. Beyond the outer plaque, there is an inner dense

plaque comprised largely of the plakin family members, including desmoplakin (Dsp), plectin, envoplakin (EVP), and periplakin (PPL) (Fig. 8b).




(a) (b)

**Fig. 8:** Structure of desmosome consisting of different anchor proteins,
**(Source: (a)** http://www.studydroid.com/index.php?page=viewPack&packId=402681,
**(b)** Ohno, 2015, http://www.sciencedirect.com/science/article/pii/S1880427616000284)

Desmosomes were first described as small, dense nodules at the contact points of adjacent cells by Bizzozero, a young Italian pathologist in 1864. Later, they were named as 'desmosomes' (from the Greek words "desmos" meaning "bond" and "soma" meaning "body") by Josef Shaffer in 1920.

The type of intermediate filaments in the desmosome differs with the cell type, for example, keratin filaments in most epithelial cells and desmin filaments in heart muscle cells. This kind of multiprotein structure, also called *desmosomal plaque* of high tensile strength provides mechanical support and is particularly plentiful in tissues, which are subjected to frequent mechanical stress like heart muscles and epidermis. Infact, the importance of desmosomes can be directly seen from the incidence of diseases like blistering of the skin, which are caused by disruption of desmosomes and other diseases tabulated in Table 1 (Gumbiner, 1996).Also, it has been reported that desmosomes can also be targeted during bacterial infection; for example, the exfoliative toxins of *Staphylococcus aureus*, which cause a number of blistering diseases, can cleave a desmosomal cadherin. Additionally, the role of desmosomes in cancer and tumor cells likes kin, head and neck, lung, breast, and a variety of other epithelial malignancies, including gastric and colon cancers has been observed with

reduced expression of cadherins resulting in loose cell-cell adhesion. Also, adaptor proteins in desmosomes regulate cell cycle and apoptosis by signaling molecules (Cirillo, 2015).

**Table 1:** Human diseases associated with mutations in proteins comprising desmosomes (Cirillo, 2015).

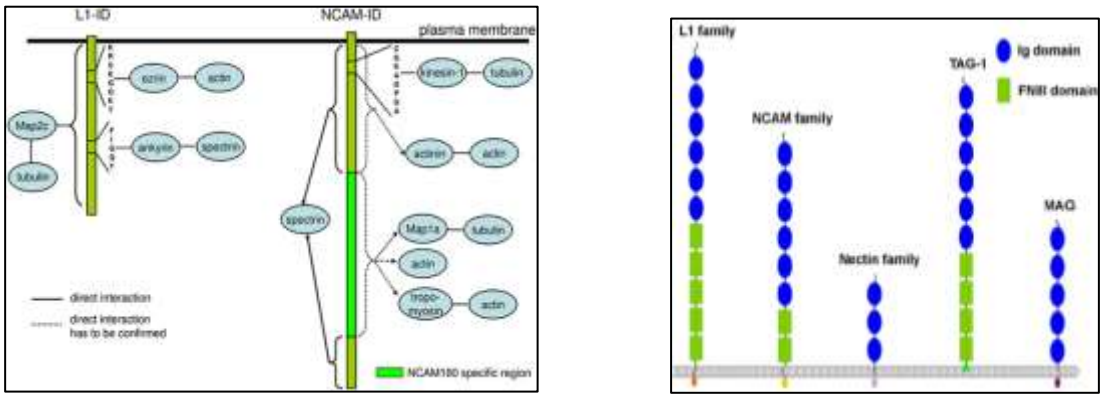
| Name of the disease/disorder | Causative mutation in gene associated with the disorder | Clinical manifestations |
|---|---|---|
| Ectodermal dysplasia–skin fragility syndrome. | Pkp1 | skin fragility (with trauma-induced erosions and blistering), alopecia, palmoplantar keratoderma (PPK), nail dystrophy |
| Striate form of Palmoplantar keratoderma (SPPK) | Dsp | abnormal thickening of the palms and soles |
| Carvajal syndrome | Dsp | SPPK, wooly hair, and dilated left ventricular cardiomyopathy, resulting in heart failure early in life |
| Non-striated form of Palmoplantar keratoderma | Dsg1 | discrete keratinization at sites exposed to mechanical trauma, such as the knees, ankles, and finger knuckles, and mild nail dystrophy |
| Hypotrichosis | Dgs4 | hypotrichosis restricted to the scalp, chest, arms, and legs, thin and atrophic hair follicles and hair shafts that often coil up within the skin due to their inability to penetrate the epidermis |
| **Other Autoimmune Diseases** | | |
| Pemphigus vulgaris (PV) | loss of desmosomes, resulting in loss of cohesion between keratinocytes (Acantholysis), autoantibodies against Dsg3 or both Dsg3 and Dsg1 | |
| Pemphigus foliaceus | superficial acantholysis in the granular cell layer of the epidermis and autoantibodies against Dsg1 | |

## 6. Immunoglobulin Super family Cell Adhesion Molecules (IgSF CAMs)

The next classes of cell adhesion molecules include the IgSF CAMs, which are transmembrane glycoproteins with a large extracellular domain consisting of immunoglobin (Ig)-like repeats and hence the name. These Ig like domains are made up of β-sheets, which

are stabilized by disulphide linkages. It is one of the largest and most diverse protein family with more than 765 members (Wong et al., 2012) and more than 50 members of this IgSF CAMs superfamily are present in mammalian nervous system, suggesting their critical role in the development of nervous system with important regulatory functions in the migration of nerve cells, growth and branching of axons and dendrites, synaptic contacts and forming neural networks between different neurons for neurotransmission. These functions contribute to the development of brain, thinking abilities, memory and movement.

Some members of this superfamily are attached to the plasma membrane by a glycosylphosphatidylinositol (GPI) anchor. Both homophillic and heterophillic interactions with other cell surface receptors as well as with the extracellular matrix, cell adhesion molecules, cytoskeletal proteins, are exhibited by IgSF CAMs. There are 5 subfamilies of IgSF CAMs, namely: L1CAM, NCAM, Nectins and Nectin-like molecules, TAG-1 and MAG (Fig. 9a).




(a) (b)

**Fig. 9 (a)**: Structure of members of IgSFS CAMs superfamily. **Source:** Bian, 2013, https://www.intechopen.com/books/neural-stem-cells-new-perspectives/cell-adhesion-molecules-in-neural-stem-cell-and-stem-cell-based-therapy-for-neural-disorders,
**(b)** Interactions of L1 & NCAM with anchor proteins which further link these CAMs to cytoskeleton.
**(Source:** Leshchyns'ka & Sytnyk, 2016, http://journal.frontiersin.org/article/10.3389/fcell.2016.00009/full)

The members of these subfamilies differ only in their EC domain, with variable number of Ig-like domain, which assist in cell-cell binding and fibronectin type III or FNIII repeats

which are involved in signaling that regulate the neurite growth. In this section, we shall discuss some of the members of this superfamily.

### *6.1. L1CAMs*

The structure of **L1CAMs**is a neural cell adhesion molecule with important functions in the development of nervous system. It consists of a transmembrane domain, cytoplasmic tail and an extracellular domain comprising of 6 Ig like domains and 4-5 fibronectin type III (FNIII) repeats. L1CAMs are linked to the cytoskeleton via ankyrin protein, which is an adaptor protein and has a binding site in the intracellular domain of L1CAMs. It can bind with the actin cytoskeleton via ezrin protein also and can directly bind with tubulin through microtubule associated protein 2c (MAP2c) (Fig. 9b).Thus it can be seen that the interaction of IgSFCAMs with the cytoskeleton requires different linker or adaptor proteins and these interactions are regulated also, for example, the binding of ankyrin with L1 is regulated by phosphorylation (Bian, 2013).

The gene encoding L1CAM is present in the long arm of X chromosome at locus Xq28 near the telomere. Mutations (>350 in no.) in L1CAM can lead to *L1 syndrome*, which is an X-linked recessive disease, found exclusively in males. It primarily affects the nervous system with an incidence of one in every 30,000 males (L1CAM mutation database, http://www.l1cammutationdatabase.info/). The clinical manifestations of this syndrome consist of impairment of neural activities and causes X-*linked hydrocephalus* (hydrocephalus; accumulation of fluids in the brain) with symptoms like brain deformities, movement problems and intellectual disability or mental retardation (U.S. National Library of Medicine, https://ghr.nlm.nih.gov/gene/L1CAM#resources). It also causes MASA syndrome, an acronym for Mental retardation, Aphasia, Spastic paraplegia, Adducted thumbs, a term coined by Bianchine and Lewis in 1974. It is characterized by a variable degree of severity, i.e. it can range from mild to moderate intellectual deficit, delayed development of speech, hypotonic progressing to spasticity or spastic paraplegia, adducted thumbs, and mild to moderate distension of the cerebral ventricles. Mutations in L1CAM also causes *Hirschsprung's disease*, which results from a defect in migration of neural crest cells in distal segments of gut with associated abnormalities such as urological disorders, dysautonomia

(conditions affecting autonomic nervous system), and structural gastrointestinal abnormalities. Aberrant expression of L1CAM is also reported in several different types of cancers, including colon carcinoma, ovarian and uterine carcinomas, malignant gliomas, recurrent neuroblastoma, cutaneous malignant melanoma, renal cell carcinoma, extrahepatic cholangiocarcinoma (ECC) and gallbladder carcinoma (Weledji & Assob, 2014).

### *6.2. NCAM*

NCAM or the Neural cell adhesion molecule is a glycoprotein, which is present on neurons, glial cells, and natural killer cells. It is also called as CD56 and has three major isoforms (formed by alternative splicing), named according to their molecular weight: NCAM-120, NCAM-140, and NCAM-180. The EC domain of NCAM consists of five Ig-like domains and two FNIII repeats and a transmembrane domain in case of NCAM-140 and NCAM-180, while a GPI anchor which links NCAM-120 to the plasma membrane (Bian, 2013). NCAMS exhibits both *trans* and *cis*-homophillic interactions on different and same cell surfaces respectively. Like LCAM, NCAM also binds with the cytoskeletal proteins, for instance, NCAM-180 binds to tubulin (via kinesin-1), actin, $\beta_1$spectrin, Microtubule Associated Protein 1A(MAP1a) and tropomyosin. NCAM is also found to be associated with axon fasciculation, promoting growth of axon in bundles in addition to its function in axonal outgrowth. The axon fasciculation is facilitated by NCAM, which undergoes post-translational modification by the addition of polysialic acid (PSA), thus forming PSA-NCAM. The addition of negatively charged PSA reduces the binding affinity of NCAM, which leads to decreased cell adhesion but increased cell migration and invasion. This is because the negatively charged sialic acid residues tend to sequester water molecules and hence occupy larger volume in extracellular space. This causes repulsion between the opposing cell membranes and keeps the adhesion molecules like cadherins and L1CAMs away from interaction. Hence, NCAM, which keeps the axon in tight association with each other, facilitates their fasciculation also by cell adhesion, while PSA-NCAM promotes axonal growth by suppressing axon fasciculation (Fig. 10).

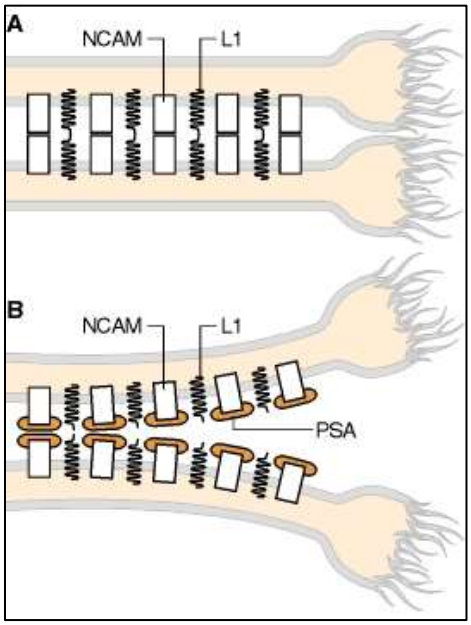



**Fig. 10:** Axon fasciculation, A: NCAM without polysialic acid is present on an axon, wherein, NCAM and L1 molecules undergo homophillic binding and axons fasciculate. B: NCAM carries polysialic acid (PSA), hence NCAM and L1 molecules cannot interact and growing axons do not fasciculate.
**(Source:** Colman & Filbin, 1999, https://www.ncbi.nlm.nih.gov/books/NBK28015/)

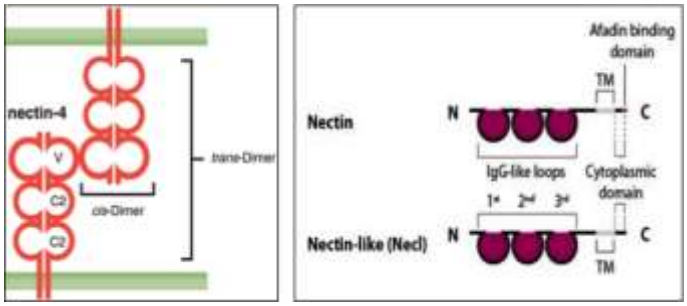
NCAM is also associated with metastasis in different types of cancer like melanoma, glioma, breast, ovarian, endometrial, prostate, and colon cancer (Wong et al., 2012).

### *6.3. Nectins and Nectin-like molecules (Necls)*

Nectins and Nectin-like molecules (Necls) form another major family of $Ca2^{+}$ independent cell adhesion molecules present in large number of tissues, including epithelia and neuronal tissues. Like other IgSFCAMs, they have an extracellular domain with three Ig-like repeats, a transmembrane domain and a cytoplasmic tail. The EC domain consists of N-terminal variable region-like (V-like) domain and two extracellular constant region-like (C-like) domains (Struyf et al., 2002, Fig. 11a). The nectin family consists of four members: nectin-1, -2, -3, and -4, with their two or three splicing variants. Nectins and Necls form cis-homodimers, followed by trans-homo dimers or trans-hetero dimers (Fig. 11b). Interestingly, the heterophillic interactions (like nectin-1 bindin with nectin-3 & nectin-4 or nectin-2 binding with nectin-3) are much stronger than the homophillic interactions. Necls have similar domain structure like nectins and both may or may not assist with each other in cell

adhesion, movement, and proliferation; for example, Necl-5 is localized at the leading edge of moving cells.

There are three types of nectin-mediated cell adhesions are observed: afadin- and cadherin-dependent, afadin-dependent and cadherin-independent, and afadin- and cadherin-independent. The third type of nectin-mediated adhesion apparatus is also named as *nectin spot* (Mizutani & Takai, 2016). All nectins, except nectin-4 have a conserved motif (Glu/Ala-X-Tyr-Val) at their C-termini (Fig. 10b), which binds with PDZ [(for PSD95–DLG1–ZO-1; Post-synaptic density protein 95 (PSD-95), *Drosophila* disc large tumor suppressor (Dlg1)] and *zona occludens* 1 (ZO-1) domain of afadin, an F-actin-binding protein (Ogita & Takai, 2006). This binding motif facilitates the interaction of nectins with cytoskeletal proteins. Such protein complexes like nectin-afradin and E-cadherin-catenin are present in epithelial cells.




(a) (b)

**Fig. 11: (a)** Structure of nectin and nectin-like adhesion molecules, extracellular N-terminal variable region-like (V-like) domain, two extracellular constant region-like (C-like) domains: C1, C2 .
Source: https://www.mechanobio.info/figure/1384242495205/,
**(b)** Structure of nectin & necls depicting afadin-binding domain,
Source: Sato et al., 2012, http://journal.frontiersin.org/article/10.3389/fmicb.2012.00075/full,

Nectins also play an important role in the organization of synapses, wherein; nectin-1 and nectin-3 are present at the presynaptic and postsynaptic sides of the plasma membrane of the puncta adherentia junctions (between nerve terminals & dendrites) respectively. Further, nectin-2, nectin-3 and afadin colocalize with the F-actin to form the Sertoli-cell-spermatid junctions, referred to as the *ectoplasmic specialization* (ES). It stabilizes the adhesive domain formed by the multiprotein complex by acting as a scaffold beneath the plasma membrane of Sertoli cells.Nectin-2 and afadin also localize at Sertoli-cell-Sertoli-cell junctions, which

serve as the `blood-testis' barrier (Takai & Nakanishi, 2003, Rikitake et al., 2012). Nectin and nectin-like proteins also regulate the functions of Natural Killer (NK) cells via another adhesion molecule called DNAM-1.NK cells are the key players of body's innate immune response against viral infection and malignant transformation. Thus, NK cells express diverse inhibitory and activating receptors in order to regulate their functioning without causing any damage to healthy cells. These receptors include DNAM-1 and T cell immunoglobulin and ITIM domain (TIGIT) (de Andrade et al., 2014, Deuss et al., 2017).

Mutations in the nectin-1 gene are responsible for an autosomal recessive disorder, called cleft lip/palate ectodermal dysplasia, also known as *Zlotogora-Ogür syndrome* & Margarita Island ectodermal dysplasia. It is characterized by cleft lip/palate (gap in the upper lip and/or the roof of their mouth), ectodermal dysplasia (large, diverse group of genetic disorders that are defined by primary defects in the development of 2 or more tissues derived from embryonic ectoderm like skin, nails, teeth, glands, etc.) and loss of intellectual ability or mental retardation. Mutations in nectin-4 also cause ecdodermal dysplasia syndactyly syndrome 1, which is characterized by hair and tooth abnormalities, alopecia or spot baldness and cutaneous syndactyly. Nectin-1 and nectin-2 have also been reported to function as entry receptors for mammalian alpha herpes viruses through interaction with viral glycoprotein D (gD) (Struyf et al., 2002).

### *6.4. TAG-1*

TAG-1(Transient Axonal Glycoprotein 1) is also the member of IgSFCAMs superfamily, which plays an important role in neurite outgrowth or extension and cell aggregation. It is a glycoprotein with a molecular weight of 135 KDa and is also known as TAX-1in humans or axonin-1. Its structure consists of five Ig-like repeats, four FNIII repeats and is anchored to the plasma membrane by a GPI tail. It is expressed by neurons and myelinating glia. It promotes cell aggregation by homophillic binding through the fibronectin repeats, though it can also mediate heterophillic interactions (Bian, 2013, Sittaramane et al., 2009).

### *6.5. MAG*

**MAG (Myelin-associated glycoprotein)** is a cell adhesion molecule that belongs to IgSF superfamily, which is expressed in central nervous system (CNS) and peripheral nervous system (PNS) on the surface of oligodendrocytes and Schwann cells respectively. There are two isoforms of MAG: small (S-MAG, 67 KDa) and large (L-MAG, 72 KDa), with an EC domain of five Ig-like repeats, a transmembrane segment followed by cytoplasmic domain. The EC domain consists of N-glycosylation sites and a fibronectin recognition sequence (arg-gly-asp), and the intracellular domain has a tyrosine phosphorylation site (https://www.omim.org/entry/159460). They are important for neuron-oligodendrocyte and oligodendrocyte-oligodendrocyte interactions in the CNS and glia-glia interaction in the PNS during the development and maintenance of myelin sheath. A homozygous missense mutation in MAG has been found to be associated with *Pelizaeus-Merzbacher* disease, which destabilizes the protein and affects its tertiary structure. It is an X-linked hypomyelinating leukodystrophy, characterized by nystagmus (a condition of involuntary eye movement), jerky head movements, hypotonia and variable movement disorders during infancy (Lossos et al., 2015).

In addition to the IgSF CAMs discussed here, there are many other IgSF CAMs, present in mammalian body systems, like intercellular cell adhesion molecule (ICAM-1, expressed on endothelial cells and cells of the immune system), vascular cell adhesion molecule (VCAM-1, expressed on blood vessels), platelet-endothelial cell adhesion molecule (PECAM-1, expressed on the surface of platelets, monocytes, neutrophils and some types of T-cells). Thus, it can be summarized that IgSF CAM superfamily comprises large number of members present in body tissues, specifically in the nervous system, paying a key role in cell-cell adhesion. These adhesion molecules have been associated with metastasis and cancer progression. These molecules have been found to be associated with each step of metastatic process, such as evasion of apoptosis, angiogenesis, followed by local invasion which is mediated by cell-cell interactions, directional cell migration, ECM degradation, dissemination, extravasation, colonization & proliferation and escape from the immunological responses (Wong et al., 2012).

## 7. Selectins

Selectins are transmembrane glycoproteins, composed of 3 extracellular domains, namely: calcium binding lectin domain (hence the name)or carbohydrate recognition domain on the N-terminus, an epidermal growth factor(EGF)-like domain, and a differing number of consensus repeats (CR) (related to complement regulatory proteins), followed by a transmembrane domain and a short cytoplasmic tail at the C- terminus (Fig. 12). There are three family members identified: E-selectin, L-selectin and P-selectin. E-selectin is expressed exclusively on endothelial cells, L-selectin is found on all lymphocytes except activated T-lymphocytes and P-selectin found in secretory granules of plateletsand endothelial cells. The EGF domain is involved in ligand recognition along with lectin domain, while complement binding repeats participate in protein binding. The cytoplasmic domain plays a role in regulating cell adhesion by cytoskeletal interactions (Ley, 2013, Leshko-Lindsay & Corces, 1997).

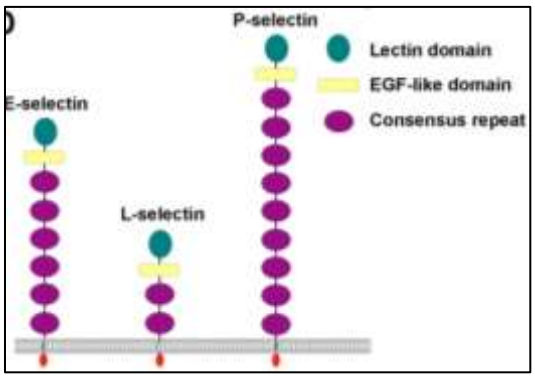



**Fig. 12:** Structure of members of Selectin family.
**(Source:** Bian, 2013, https://www.intechopen.com/books/neural-stem-cells-new-perspectives/cell-adhesion-molecules-in-neural-stem-cell-and-stem-cell-based-therapy-for-neural-disorders)

Selectins bind to ligands expressed on activated leucocytes, which include carbohydrate epitopes, glycans belonging to the Lewis family of glycoepitopes, like Sialyl Lewis X (sLeX, glycans that consist of fucose linked to the GlcNAc monosaccharide), CD44, Cutaneous lymphocyte associated antigen (CLA) and P-selectin glycoprotein ligand 1 (PSGL-1). Selectins play a very important role in innate adaptive immunity, like E-selectin recruits leukocytes including monocytes, neutrophils, and lymphocytes to the site of inflammation.

The development of an inflammatory response in conditions like diabetes, atherosclerosis, rheumatoid arthritis and cancer, is a highly regulated process, which involves adhesion and rolling of circulating leucocytes along the vascular endothelial cells by interaction of E-selectin and its ligand, followed by transendothelial migration into the inflamed tissue (Fig. 13). Now, E-selectin is not expressed on endothelial cells under normal conditions but its expression is induced rapidly due to stimulation by cytokines: tumor necrosis factor α (TNF-α) and interleukin (IL)-1β, which act as transcription factors with binding sites in E-cadherin gene. Also, fibroblasts associated with chronic inflammation can directly induce lymphocyte adhesion (Mann & Tanaka, 2011, Mollà & Panés, 2007).

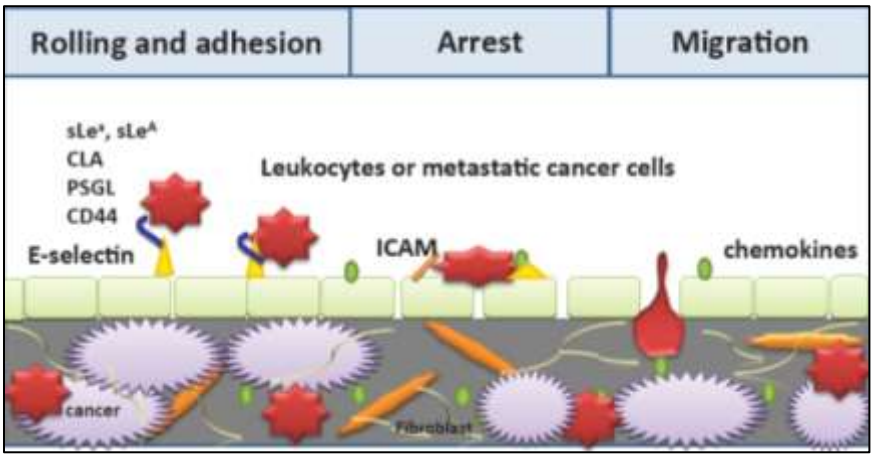


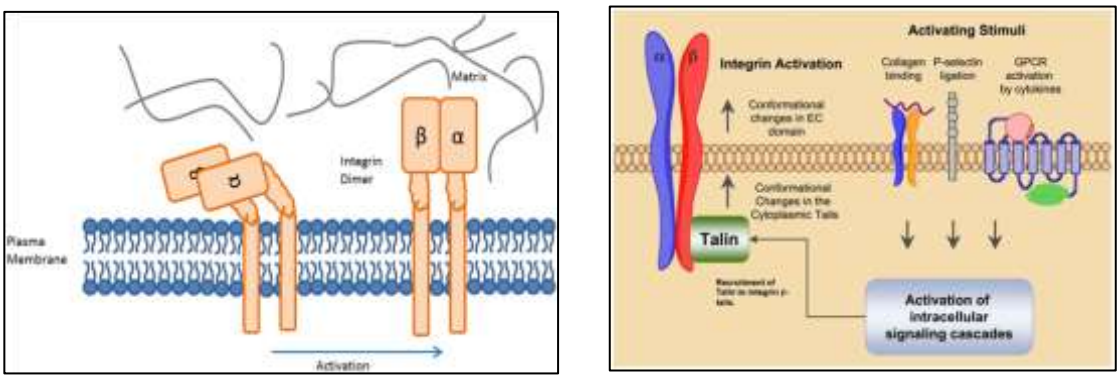

**Fig. 13:** E-Selectin mediated transendothelial migration of leukocytes during inflammation. In the first step, leucocytes or metastatic cancer cells attach and roll along the vascular endothelial cells (rolling and adhesion) mediated by interaction of E-selectin with its ligand. In the second step (arrest), the cells adhere tightly to the endothelial cells and spread over the endothelium and actively transmigrate through the endothelial lining. Chemokines regulate this step, activates integrins, and facilitates firm adhesion through ICAM and VCAM for subsequent transmigration across the endothelial lining.
**(Source:** Mann & Tanaka, 2011, https://www.omicsonline.org/e-selectin-its-role-in-cancer-and-potential-as-a-biomarker-2161-1025.S1-002.php?aid=2339)

In addition to leucocyte transmigration during inflammation, L-selectins along with their oligosaccharide ligands (MECA-79) have been reported to play an important role in establishment of embryo-maternal interactions in case of humans. L-selectins are expressed on the external surface of human blastocysts and hence mediates in adhesion of blastocyst with the endometrial epitheliumor implantation of embryo in the uterus lining (Nejatbakhsh et al., 2012).

## 8. Integrins

Integrins are heteromerictrans membrane glycoproteins, which constitute a large family of cell adhesion molecules. Infact, the term was used Hynes in 1987 in his review article to describe a family of structurally and functionally related cell-surface heterodimeric receptors, which "*integrated"* the extracellular matrix with the cellular cytoskeleton to mediate cell migration and adhesion.

The structure consists of two different subunits: α and β which are joined non-covalently to each other. Both the subunits have a short intracellular tail of ~40-70 amino acids in length. The EC domains of both subunits bind to ligands in divalent cations ($Ca^{2+}$, $Mg^{2+}$ or $Mn^{2+}$) dependent manner. Integrins recognize RGD (Arg-Gly-Asp) sequence present in ECM proteins (like fibronectin, vitronectin, collagen and laminin), thereby facilitating cell-ECM interactions, which further play important role in various biological functions like cell growth, survival, and differentiation (Roggiani et al., 2016, Shishido et al., 2014, Millard et al., 2011).The integrins are able to exert their function by the binding of ligands (extracellular ligands of integrins; the listing is undoubtedly incomplete. The list includes a large number of extracellular matrix proteins (bone matrix proteins, collagens, fibronectins, fibrinogen, laminins, etc.), which also stabilize its conformation from a bent to an extended conformation (Fig. 14a).




(a) (b)

**Fig. 14 (a):** Conformational change in structure of integrin upon ligand binding,
**(b)**: Activation of integrins by chemokine signaling and mechanical stress
**Source:** Shishido et al., 2014,http://journal.frontiersin.org/article/10.3389/fonc.2014.00099/full

In addition to ligand binding, other extracellular factors that affect activation of integrins includechemokine signaling and mechanical stress (Fig. 14b). The resulting intracellular signaling induces conformational changes in the tail of beta subunit of integrin, which further mediates the localization of talin to the activated integrin β-tails. Talin in turn interacts with other cytoskeletal proteins like vinculin and actin, thus promoting focal adhesion.

## 9. Summary

This chapter presents a comprehensive and recent knowledge on the cooperation of cell adhesion molecules or CAMs, which are a diverse group of transmembrane proteins and play an important role in many fields like immunology, developmental biology, and malignancy. In fact, they have been referred to as "*glue of life*", maintaining integrity of tissues and organs by cell adhesion and mediating cellular communication along with junction channels. The cell-cell adhesion is required for cell migration, differentiation, developmental pathways, wound healing and regeneration and in pathological conditions like metastasis, inflammation, etc. Also, CAMs have been the targets of therapeutic research to find out cure and management of various human diseases. Thus, by unraveling the mysteries and complexities of biological communication, we can understand the basis of life as well as gain a better knowledge about major human diseases like cancer.