https://dx.doi.org/10.4314/dujopas.v7i4a.14
ISSN (Print): 2476-8316
ISSN (Online): 2635-3490
Dutse Journal of Pure and Applied Sciences (DUJOPAS), *Vol. 7 No. 4a December 2021*

Role of endothelial cell junctions in transendothelial cancer cell migration. A review

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Abstract

During metastasis, tumour cells must become migratory and travel towards a capillary within the tumour. They then degrade the matrix surrounding the pericytes and endothelial cells, insert themselves between endothelial cells, transverse the capillary wall, to then enter the blood stream. This process depends on the motile behaviour of the tumour cells as well as the role of endothelial cell-cell junctions, both including adherens junctions and tight junctions. Circulating tumour cells must next, adhere to the walls of the capillary at the site of secondary tumour formation. Here, they again traverse the capillary wall to enter tissues distant from the primary tumour. This review aim to discuss the basic architecture of the endothelial junctional complex as well as the role played by these components towards the transendothelial migration of cancer cells from the primary site to the secondary site. Proper understanding of the role played by each of these components could invariably lead to the development of novel adjuvant cancer chemotherapy.

Key words: Endothelial cells, Tight junctions, Gap junctions, Adherens junction, Metastasis.

INTRODUCTION

Endothelium refers to the monolayer single sheet of endothelial cells lining the inner aspect of the vascular lumen acting to separate the underlying tissues from the circulating blood (Chistiakov, Revin, *et al*., 2015; Curry & Adamson, 2010), thereby forming a form of barrier for the passage of both macromolecules as well as blood cells from the circulation into the underlying tissues (Dejana *et al*., 1995; Dejana & Vestweber, 2013). These barriers are made up by the endothelial cells through the interactions of various junctional structures, which acts to tightly regulate the endothelial barrier (Razakandrainibe *et al*., 2013). Two major types of intercellular junctions found to be associated with the ECs are the tight and adherens junctions (Bazzoni & Dejana, 2004; Hirase & Node, 2012). Dysfunctions or distortion of endothelial cell-cell junctions by the tumours cells results in opening of these junctions leading to

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transendothelial migration of the tumour cells at a far distant sites. which now becomes the secondary site. This review focus on describing the role played by each of these intercellular junctional complexes components during the process of transendothelial migration of the cancer cells.

An overview of general architecture of vascular system

The vascular system, also known as the cardiovascular system is a system that is concerned with the distribution/circulation of blood as well as nutrients to and from the cells of the body. The vascular system is generally made up of the blood and lymphatics. The vascular wall is made up of three distinct layers known also as tunics. Inside out are the tunicaintima, tunica media and tunica adventitia.

The tunica intima has an endothelial cell layer lining the luminal cavity of the vessel and just beneath the Endothelial cells (EC) is the basal lamina onto which the EC lies. Next to the basal lamina is the sub-endothelial layer which is made up of elastic fibres hence referred to as the internal elastic membrane. Tunica media which is the middle vascular layer is made up of vascular smooth muscle cells as well as some interspersed elastic fibres i.e. depending on the type and size of vessel. Next to the smooth muscle layer is the external elastic membrane demarcating the tunica media from the tunica adventitia. The tunica adventitia is the most external coat and by and large, it is composed of connective tissue. (**Figure 1**).

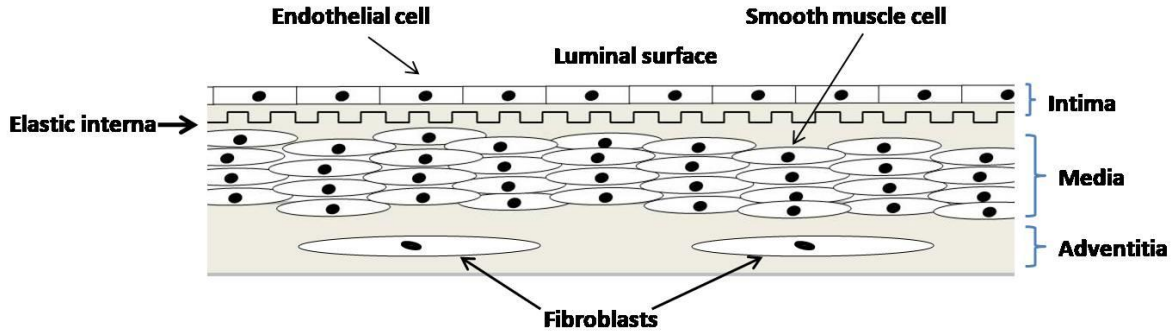

Figure 1. A cartoon diagram illustrating the three basic parts of a blood vessels. From the luminal aspect is the tunica intima followed by tunica media lying in between the tunica media and tunica adventitia. The outermost layer is the tunica adventitia.

Endothelial cell architecture

The vascular endothelium is made up of a single thin sheet layer of endothelial cells, which are flattened, elongated and polygonal in shape (simple squamous). The EC lines the entire inner aspect of the vascular system as earlier stated, as such forming a continuous inner layer. This layer of the endothelium in man has a surface area of 350 m^2 and a total mass of 110 g (Pries & Kuebler, 2006; Pries *et al*., 2000). The endothelium via the EC is actively involved in several important functions including, maintenance of selective permeability barrier, haemostasis and coagulation, inflammatory responses, fluid and solute exchange among others (Pries & Kuebler, 2006; Pries *et al*., 2000). All the EC lie on the basal lamina. The EC along with tumour cells and most other cell types possess three forms of cytoskeletal components which are always in constant and close interaction with one another, through which they establish as well as regulate the endothelial barrier functions. These EC cytoskeletons are the actin microfilaments, intermediate filaments and microtubules (Chang & Goldman, 2004; Dudek & Garcia, 2001; Revenu *et al*., 2004).

The actin microfilaments on average are about 7 nm in diameter with intermediate filaments measuring about 10 nm and microtubules being about 25 nm in diameter (Ishikawa *et al*., 1968). Inside the EC actin constitutes about 5–15% of the total proteins (Patterson & Lum,

2001). Actin cytoskeleton exhibits a dynamic nature of polymerization and depolymerization. Polymerization of β-actin and γ-actin globular (G) subunits results in the formation of filamentous (F) actin. The G-actin and F-actin are found to be in an equal amount within the cell (Stossel *et al*., 1985). However, the F actin is the main structural unit for the formation of actin-based cytoskeletal structures. The F actin cytoskeleton is composed of three distinct subtypes, comprising of cortical actin rim, outer membrane skeleton, with cross-linking of spectrin and the cytosolic actomyosin-based stress fibres (Prasain & Stevens, 2009). The F-actin filaments of the cortical actin are longer than the filaments of the membrane skeleton and stress fibres (De Matteis & Morrow, 2000; Heimann et al., 1999). In cultured endothelial cells, the actin stress fibres generate centripetal tension in addition to the reorganization of adhesion complex architecture, which eventually results to retraction of cell-cell borders into apparent gaps (Dudek & Garcia, 2001; Phillips et al., 1989). The cortical actin rim subsequently generates an outwards centrifugal tension to counteract the tension generated by stress fibres so as to prevent the collapse of the cultured motile cells. The cytosolic actomyosin-based stress fibres are necessary for cell contraction (Hotulainen & Lappalainen, 2006) during which they determine the rate and size of the inter-endothelial cell gaps (Dudek & Garcia, 2001; Patterson & Lum, 2001). Therefore, the endothelial contractile machinery consists of actin and non-muscle myosin, which requires ATP, calcium and calmodulin in order to generate contractile force for centripetal tension (Dudek & Garcia, 2001; Surapisitchat & Beavo, 2011). The endothelial cell contraction is generated sequel to process of phosphorylation of myosin light chain (MLC) by MLC-kinase (MLCK), which occur either as mono-phosphorylation on Ser19 or di-phosphorylation on both Ser19 and Thr18 (Goeckeler & Wysolmerski, 1995; Prasain & Stevens, 2009). MLCK, a calcium / calmodulin- dependent enzyme (Mehta & Malik, 2006), in the human endothelial cell is a protein with a molecular weight of 214 kDa, and present on chromosome 3 (Dudek & Garcia, 2001). Attenuation of MLC kinase activity results in distortion of endothelial barrier function (Garcia *et al*., 1995). Wainwright and colleagues reported increased protection in lung vascular permeability following the injection of lipopolysaccharide in endothelial cell MLCK -/- mice (Wainwright *et al*., 2003).

The intermediate-sized filaments have an average diameter of about 10nm (Ishikawa *et al*., 1968). The intermediate filaments of the EC are constituted by the polymers of cytokeratin and also vimentin(Patton *et al*., 1990). The filaments possess the ability to extend their length from both of the ends (Mehta & Malik, 2006). The intermediate filaments in epithelial cells play an important role in cell-cell junctions and cell-ECM junctions at the desmosomes and hemidesmosomes through the interactions of transmembrane desmoglein and desmocollin proteins. Desmoglein and desmocollin are members belonging to the cadherin family (Garrod & Chidgey, 2008; Mechanic et al., 1991). Unlike epithelial cells, ECs do not possess desmosomes (Lampugnani & Dejana, 1997; Rubin, 1992) rather, the EC vimentin is usually linked to the adheren junction structure which is similar to the desmosomes, thereby forming an adherens complex (complexus adherens) (Kowalczyk et al., 1998; Schmelz & Franke, 1993; Schmelz et al., 1994; Valiron et al., 1996). In addition, vimentin through desmoplakin, an intermediate filament-binding protein is able to form a link between the adheren junction and intermediate filaments of the EC (Shasby *et al*., 2002). Also, an alternative link between AJ and intermediate filaments could be established through the desmoplakin binding to the AJ armadillo protein p0071 (Calkins et al., 2003; Valiron et al., 1996). During inflammation, mediators are capable of disrupting the normal interaction between intermediate filament, γ-catenin as well as the VE-cadherin (Shasby *et al*., 2002). However, vimentin knock-out mice lacking endothelial cell intermediate filaments did not exhibit any structural abnormality in their EC (Colucci-Guyon *et al*., 1994).

Microtubules are rigid hollow polymer tubes of tubulin formed through the organisation and assembly of the α and β tubulins heterodimers, which originates from the microtubule-organizing center (MTOC) close to the cell nucleus to then spread up to the periphery of the cell (Prasain & Stevens, 2009; Wade & Hyman, 1997). Each of the tubulins is about 55 kDa(Chretien & Wade, 1991). As the microtubules extend throughout the cytosol it utilizes the motor proteins dynein and kinesin thereby providing a good platform which allows intracellular transport of proteins throughout the cell (Krylyshkina *et al*., 2002; Lambert *et al*., 1997; Wade & Hyman, 1997). Microtubule has an outer diameter of 25 nm while its inner diameter is 12 nm (Wade & Hyman, 1997). The α and β tubulins subunits polymerize in a head-to-tail fashion thereby forming protofilaments which is subsequently aligned lengthwise along the microtubule. The protofilaments formed associate laterally to form a single microtubule having a hollow space measuring 12 nm in diameter at the inner aspect (Kikkawa *et al*., 1994). The wall of a single microtubule, therefore, is made from approximately 13-14 parallel protofilaments (Chretien *et al*., 1992; Kikkawa *et al*., 1994). Thus these rigid hollow rods assists EC in resisting compression through opposing actin-myosin contractility (Mehta & Malik, 2006). The microtubule, unlike the intermediate filaments, displays a distinct polarity at their ends. These are the plus (+) and minus (-) ends. The plus end have the β tubulins subunit exposed. It is the faster-growing end, found to be attached to the cortical actin layer on the inner face of the plasma membrane, and elongation is faster at this end compared to the minus end which has α tubulin subunit exposed (Grego *et al*., 2001; WatermanStorer & Salmon, 1997). The exposed β tubulin at the plus end binds to and hydrolyses GTP to GDP, thereby allowing dynamic treadmilling of the microtubule, while the exposed α tubulin does not hydrolyse its attached GTP (Grego *et al*., 2001; WatermanStorer & Salmon, 1997). It is important to note that though microtubules do not extend to the plasma membrane (Conacci-Sorrell *et al*., 2002; Dejana, 2004; Dudek & Garcia, 2001; Lampugnani *et al*., 2002) and also do not directly interact with cellular junctions (Vincent *et al*., 2004), they are capable of delivering cell junction components to the cell surface through the vesicular trafficking of p120 catenin, a component of AJ as well as the delivery of connexin hemi-channels for the gap junction channels (Chen *et al*., 2003; Lauf *et al*., 2002; Shaw *et al*., 2007; Yanagisawa *et al*., 2004). Microtubules are always in a dynamic state of assembly and disassembly and initially, microtubules and actin filaments were regarded as individual entities. However several studies have revealed an intricate association between microtubule and actin (Fuchs & Yang, 1999; Goode *et al*., 2000; Klymkowsky, 1999; Prasain & Stevens, 2009). The interaction between microtubule and actin could be direct via the microtubule-associated proteins or indirectly via the intermediate linker or scaffolding proteins (Goode *et al*., 2000; Lee & Gotlieb, 2002; Lee & Gotlieb, 2003), including coronin (Goode *et al*., 1999), centractin (Clark & Meyer, 1992) and IQGAP1 (Brunner, 2002; Fukata *et al*., 2002) which bind to both microtubules and actin. The mechanism of IQGAP1 is well understood. The IQGAP1 cross-links via its amino terminus with F actin and then provides a carboxyl terminus as the binding site for microtubule-binding proteins CLIP-170 and EB1 (Brunner, 2002; Fukata *et al*., 2002; Watanabe *et al*., 2004). Both CLIP-170 and EB1 are microtubule plus end protein (Gundersen, 2002; Schuyler & Pellman, 2001). Also, EB1 has dual binding sites at its C-terminus for p150, is a component of the dynein / kinesin complex (Askham et al., 2002; Berrueta et al., 1999). In addition β-catenin, which is a component of AJs, could also bind to dynein thereby indirectly linking the AJ to the plus end of microtubule and therefore is suggested to link the microtubule plus-end to AJs (Chausovsky *et al*., 2000; Komarova *et al*., 2000; Ligon *et al*., 2001). Furthermore, disruption of microtubule like the disruption of the cortical actin network has been reported to impair the endothelial cell barrier function. Microtubule disruption activates Rho guanine exchange factors (RhoGEFs), from tubulin, resulting in Rho A activation and subsequent stress fiber formation (Krendel et al., 2002; van Horck et al., 2001), while polymerisation of microtubule sequesters LIM kinase 1 thereby, restricting its access to the actin cytoskeleton and preventing

the formation of stress fibres (Gorovoy et al., 2005; Maekawa et al., 1999). Interestingly chemotherapeutic agents such as vinca alkaloids were reported to cause pulmonary oedema secondary to microtubule disruption (Cattan & Oberg, 1999). Also, inhibition of the motor protein activity of kinesin-1 or treatment using a depolymerising agent such as nocodazole has been shown to cause increased endothelial permeability (Verin *et al*., 2001). Thus, microtubule proves to be critical for the maintenance of cell shape as well as barrier integrity of the endothelium.

Endothelial cell-cell contacts and junctions

The ECs along the vascular tree maintain contact with each other through intercellular junctions. The barrier function of the ECs is known to be highly mediated by these junctions, in addition to keeping the ECs together. The architecture of the junctions along the vascular system is highly heterogeneous and depends on specific requirements of a particular organ (Engelhardt & Wolburg, 2004; Wolburg & Lippoldt, 2002). The intercellular junctions are highly dynamic and as such play an important role in the maintenance of vascular homeostasis as well as in inflammation during the transendothelial migration of leukocytes. Disruption of these junctions in disease conditions including cancers often leads to the breakdown of the EC barrier, increasing the permeability (Engelhardt & Wolburg, 2004; Goswami & Vestweber, 2016). At least three main types of intercellular junctions have been described in endothelial cells: Tight junctions (TJ), Adherens junctions (AJ) and Gap junctions (GJ) as illustrated in the Figure 2. These junctions are briefly described below.

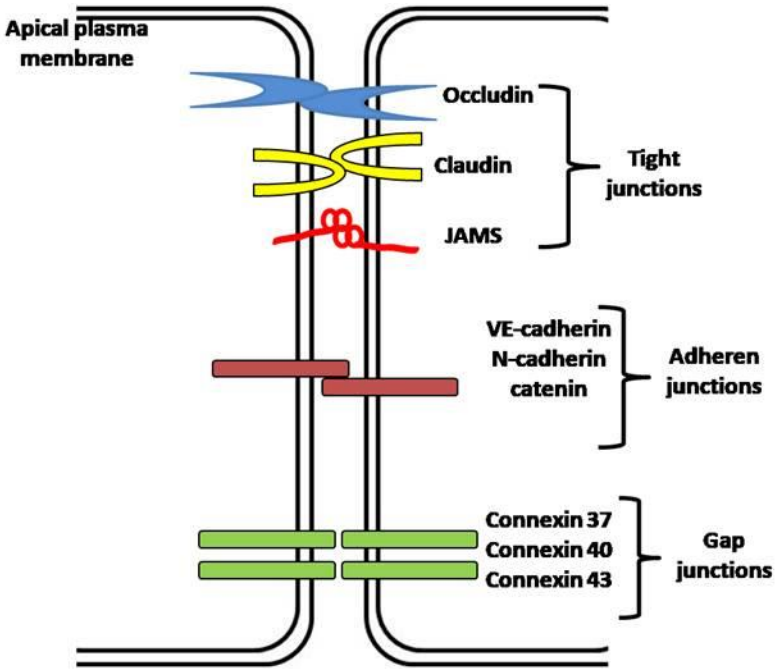

Figure 2. Endothelial cells junctions. The figure illustrates the main types of junctions present in vascular endothelial cells, tight junctions, adherens junctions and gap junctions. Tight junctions are usually the most apical in epithelial cells and less restricted in endothelial cells. TJ components include occludin (blue), claudin (yellow) and junctional adhesion molecules (JAMs) (red). Adherens junctions in endothelial cells are formed mainly by VE-cadherin. Also, N-cadherin is found to be expressed by ECs. Gap junctions in endothelial cells are formed from connexin proteins, mainly connexins 37, 40 and 43. These junctions are paracellular channels responsible for the intercellular exchange of ions as well as small molecules.

Tight junctions

Tight junctions (TJ) are found to be present in both epithelial and endothelial cells. TJ was identified as a specialization of the plasma membrane via electron microscopy in 1963 (Farquhar & Palade, 1963). Freeze fracture study shows the TJ to be composed of a network of linear fibrils intersected by short transversal fibrils (Anderson *et al*., 1993; Gumbiner, 1993). The TJ in ECs, unlike those in epithelial cells, is less structured and more intermixed with the adherens junctions. They are found to be the most apical amongst all the junctional complexes in both epithelial and endothelial cells (Bazzoni & Dejana, 2004; Chistiakov, Orekhov, *et al*., 2015). They provide both barrier or gate function through regulation of entry and exit of ions, water, and macromolecules between cells (paracellulary), as well as fence function thereby establishing and maintaining the polarity of the cell (Bazzoni & Dejana, 2004; Chistiakov, Orekhov, *et al*., 2015; Dejana, 2004; Tsukita *et al*., 2001). The distribution of TJ varies along the vascular network and depends largely on the requirement for endothelial permeability (Simionescu & Simionescu, 1991). The endothelium of large arteries tends to have a well-developed tight junction system while TJs are absent in post capillary venules (Aird, 2007; Simionescu & Simionescu, 1991; Wallez & Huber, 2008). Additionally, TJs are found to be well developed in brain and retina where they form the blood-brain barrier and blood-retinal barrier, respectively (Simionescu *et al*., 1975; Wallez & Huber, 2008). Finally, recently the loss of TJ barrier function have been described to play a role in cancer metastasis (Martin, 2014; Martin & Jiang, 2009). In ECs as well as epithelial cells the TJs are formed by the homophilic interaction of cell-cell adhesion molecules, which are membrane-associated proteins. These include claudins, occludins, and junction adhesion molecules (JAMs) (Balda & Matter, 2016; Ebnet, 2008; Radeva & Waschke, 2018).

Claudins, a family of proteins are the major barrier-forming proteins in TJs (Tsukita & Furuse, 1999; Tsukita *et al*., 2019). They comprise of more than 20 members, with a low molecular mass of 20–27 kDa. Claudin members are capable of establishing both homophilic binding and heterophilic binding (Furuse & Tsukita, 2006; Runkle & Mu, 2013; Tsukita & Furuse, 1999). Claudin, like occludin, possesses four membrane-spanning regions, thus referred to as a tetraspan protein, with two extracellular loops and two cytoplasmic termini (N- and C-termini) (Bazzoni & Dejana, 2004; Chiba *et al*., 2008; Van Itallie & Anderson, 2014). The cytoplasmic C-terminus of claudin bears a PDZ motif through which it recruits PDZ scaffolding proteins (Hamazaki *et al*., 2002; Itoh *et al*., 1999; Roh *et al*., 2002). ECs in humans have been reported to express claudin-1, −3, −5, −12, and −15 (Chistiakov, Orekhov, et al., 2015; Kiuchi-Saishin et al., 2002; Morita et al., 1999; Witt et al., 2003). Recent studies have reported an increase in TJ permeability following knockdown of claudin-1 in human ECs (Asaka *et al*., 2011). Additionally, Claudin-5-knockout mice often have developed severe brain haemorrhage due to selective impairment in BBB function for molecules smaller than 800 Da (Nitta *et al*., 2003; Runkle & Mu, 2013). Taken together the above evidence points to the key role of claudin-1 and claudin-5 in the regulation of endothelial TJ permeability.

Occludin was the first transmembrane protein to be discovered in tight junctions (Furuse *et al*., 1993). It has a molecular mass of 65-kDa. It is also a tetraspan protein, with two extracellular loops and two cytoplasmic termini (N- and C-termini) (Furuse *et al*., 1993). Occludin through its C- terminus binds several proteins within the cytoplasm including zona occludens (ZO), in this manner mediating the interaction between the adhesion molecules and actin filaments (Balda & Matter, 2016; Balda et al., 1996). Additionally, ZO also binds to the adherens junction proteins afadin and α-catenin (Itoh et al., 1997; Ooshio et al., 2010; Rajasekaran et al., 1996). Although claudins are essential for TJ formation, in contrast, occludin is not essential, but rather serves as a major component for the formation of TJ complexes in the presence of claudins (Furuse *et al*., 1998). Occludin plays an important role in maintaining the stability and barrier function of the tight junctions (Furuse *et al*., 1993; Schneeberger &

Lynch, 2004). Occludin is known to be entirely localized to the tight Junctions of both epithelial and endothelial cells and its expression in the endothelium largely correlates with the permeability function along the vascular network (Anderson *et al*., 1993; Chiba *et al*., 2008). Additionally, occludin is found to be highly expressed brain endothelial as well as in retinal endothelial cells where they form BBB and BRB respectively (Hirase *et al*., 1997). Down-regulation of occludin has been reported to be associated with disease conditions affecting BBB and BRB (Brown & Davis, 2002). The TJ of ECs in occludin-null mice were intact and devoid of any gross alterations (Saitou *et al*., 2000), however these mice demonstrated several abnormal phenotypes including postnatal growth retardation, thinning of compact bone, calcification in the brain, testicular atrophy, male infertility, loss of cytoplasmic granules in salivary epithelial cells, females not suckling their young, and gastric inflammation and hyperplasia (Saitou *et al*., 2000). These suggest the complexity associated with occludin functions and also its role in stabilisation of the tight junctions.

Junctional adhesion molecules (JAMs), unlike the previous two proteins already described, are a family of single membrane-spanning proteins with an extracellular domain of two IgG-like folds, a transmembrane domain, and a cytoplasmic tail (Martin-Padura *et al*., 1998). The JAMs are subdivided into two subgroups, with JAM-A, JAM-B and JAM-C belonging to the first subgroup (Ebnet et al., 2003; Ebnet et al., 2001; Itoh et al., 2001) and coxsackie and adenovirus receptor (CAR), endothelial cell-selective adhesion molecule (ESAM) and JAM4 as the members of the second group (Kansaku et al., 2006; Sollerbrant et al., 2003). The first subgroup has a class II PDZ domain-binding motif at their C-terminal ends, through which directly interacts with ZO-1 and PAR-3 (Ebnet et al., 2003; Ebnet et al., 2001; Itoh et al., 2001), whereas the second subgroup bears class I PDZ domain-binding motif at their C-terminus (Bazzoni, 2003; Chiba et al., 2008; Ebnet et al., 2004; Kansaku et al., 2006). JAMs are capable of establishing both homophilic and heterophilic adhesion through their extracellular domains (Bazzoni, 2003; Ebnet *et al*., 2004). In addition to being present in TJ of EC cells, JAMs are also found in other cells including leucocytes where they play an important role in their transendothelial migration (Ebnet *et al*., 2004). JAM-A is to be found in intercellular junctions between epithelial and endothelial cells, as well as on the membranes of platelets and leukocytes (Martin-Padura *et al*., 1998; Williams *et al*., 1999), JAM-B expression is, however, restricted to inter-endothelial junctions, mainly in post capillary endothelial cells and lymphatic vessels (Palmeri *et al*., 2000). Though JAM-C is expressed in endothelial cells, its tissue distribution, by and large, varies between mouse and human (Morris *et al*., 2006). In JAM-A $^{-/-}$ mice, antibody treatment using anti-JAM-A antibody was shown to have no effect on the transendothelial migration of neutrophils (Corada *et al*., 2005; Khandoga *et al*., 2005). Recently Seung-Eon and colleagues reported a reduction in the transendothelial migration of bone marrow-derived dendritic cells following treatment with a junctional adhesion molecule (JAM)-Like (JAML) antibody (anti-JAML) (Roh *et al*., 2018). The above evidence demonstrates the important role played by JAMs in TEM, thus taken together JAMs could serve as a therapeutic target for metastasis control in neuroblastoma patients.

Gap junctions

Gap junctions (GJs) are composed of clusters of intercellular channels largely formed through the hexameric assemblies of connexins, which are located between adjacent cells (Goodenough *et al*., 1996). Two hexameric connexins from adjacent cells interact with each other through the narrow extracellular gap of about ~2 nm, from which the junction derives its name (Yeager & Nicholson, 1996). The GJs in addition to providing direct cell-cell communications also allows the exchange of ions as well as small signalling molecules (~< 1 kDa) between adjacent cells (Carter & Ogden, 1994; Evans *et al*., 2006; Hsiao *et al*., 2010; Laird, 2006). In chordates, the connexins are made up of 21 family members (Alexopoulos *et al*., 2004;

Cruciani & Mikalsen, 2007). Amongst the 21 known connexins, Cx37, Cx40, and Cx43 are the main connexins identified to be expressed by the endothelial cells within the human vascular network (Evans & Martin, 2002; vanRijen *et al*., 1997; Yeh *et al*., 1998). In the beginning, the channel function of gap junctions was mainly ascribed to the functional activity described as gap junction intercellular communication (GJIC) (Kotini & Mayor, 2015; Simon & Goodenough, 1998). Apart from their channel function, gap junctions additionally play a role in cell-cell adhesion through an association with the tight junctions (Evans & Martin, 2002; Severs *et al*., 2001). This function is largely carried out by the cysteine residues of the extracellular loops. Previous studies reported an increase in the adhesive ability of glioma cells due to an exogenous expression of connexin (Lin *et al*., 2002). Similarly, a reduction in cell-cell adhesion was observed following a point mutation in a cysteine residue of the extracellular loop (Lin *et al*., 2002). Additionally, in cancers, high expression of Cx26 was detected on the plasma membranes of mouse skin cancer cells invading the lymph node (Kamibayashi *et al*., 1995), and a similar finding was reported in the prostate as well as in breast cancer (Kanczuga-Koda *et al*., 2006). Recently, in the light of a possible role in cancer metastasis, Zhang and colleagues showed that inhibition of the highly expressed Cx43 in multiple myeloma cells using 18 a-glycyrrhetinic acid, a blocker for Cx43, markedly decreased adhesion and migratory capabilities of multiple myeloma cells (Zhang *et al*., 2015). Another study demonstrated an up-regulation of Cx43 in the region of contact between the tumour cell and endothelial cell (Elzarrad *et al*., 2008). Finally, pieces of evidence are beginning to emerge for the role of Cx43 in facilitating cancer cell intravasation and extravasation as revealed by the experiment using 4T-1 mouse breast cancer cell lines that eventually metastasises to the brain. In this study, up-regulation of connexin-43 was shown to be associated at the regions of tumour cell-endothelial cell contact both *in vitro* and *in vivo*, as well as in regions of intra-tumour blood vessels and micro-metastatic foci (Elzarrad *et al*., 2008; Stoletov *et al*., 2013). By and large, the preceding evidence points to the roles of gap junctions in adhesion as well as in metastasis suggesting that gap junction could serve as a therapeutic means for metastasis.

Adherens junctions (AJs)

AJs are found to be widely distributed along the vascular network. These junctions are made up of transmembrane spanning proteins which are found to be expressed by the endothelial cells of both blood and lymphatic vessels (Bazzoni & Dejana, 2004; Dejana *et al*., 1995). The transmembrane spanning adhesion proteins in AJs are known to be of the cadherin family (Aberle *et al*., 1996; Angst *et al*., 2001a, 2001b; Gumbiner, 2000; Yagi & Takeichi, 2000). Of the >20 members of the cadherin superfamily, the endothelial cells specifically express vascular endothelial (VE)-cadherin (Dejana *et al*., 1995). Additionally, during embryonic development, (VE)-cadherin was also found to be expressed in cytotrophoblastic cells as well as in the cells committed to the endothelial lineage (Breier *et al*., 1996; Fraser *et al*., 2003; Kim *et al*., 2005).

Cadherins were primarily identified within AJs through immunoelectron microscopic study in 1984 (Volk & Geiger, 1984). These cell-cell adhesion molecules are of 120-140 kDa molecular mass (Geiger & Ayalon, 1992) and they mediate their function in a Ca^{2+} dependent manner (Volk & Geiger, 1984, 1986). Cadherins, a single transmembrane glycoprotein, act through the formation of homotypic adhesive complexes with neighbouring EC(Steinberg & McNutt, 1999). Structurally, classical cadherins are characterized by the presence of a long extracellular domain, a transmembrane domain and a short cytoplasmic domain **(Figure 3)**. The extracellular region consists of the N-terminus and 5 extracellular repeats, EC1-EC5 (Lampugnani et al., 1992; Liaw et al., 1990). These 5 extracellular repeats (EC) are further subdivided into two groups; EC1-3 are inter-homologous ectodomains, each approximately 110 amino acids long, while EC4 and EC5 are less homologous repeats (Takeichi, 1990). The

extracellular domains are highly important in determining the specificity of the cadherin interactions (Ivanov et al., 2001; Lampugnani et al., 1992; Liaw et al., 1990). Each EC repeat expresses two putative calcium-binding sites (Ozawa et al., 1990; Ringwald et al., 1987), and modification in form of a single amino acid substitution at the calcium-binding site is adequate to eliminate the adhesive function of the molecule (Ozawa *et al*., 1990). The cytoplasmic domain of cadherins interacts with the cytoplasmic proteins including catenins, which, function as an intermediate linker between the cadherins and actin filaments (Ozawa *et al*., 1990; Rimm *et al*., 1995; Takeichi, 1995). Functionally, the cytoplasmic domain comprises of the juxtamembrane domain, which is responsible for cadherin-p120 catenin and cadherin-p0071 interactions, and the COOH-terminal domain which interacts with either β-catenin or γ-catenin in a discordant manner (Bazzoni & Dejana, 2004; Zhurinsky *et al*., 2000). As mentioned earlier, vascular endothelial (VE)-cadherin (also known as cadherin-5 and CD144) is the main cadherin expressed in endothelial adherens junctions (Lampugnani *et al*., 1992) and is structurally similar to the classical cadherin as described above. Thus the (VE)-cadherin acts to modulate the permeability of the endothelial barrier via the activity of AJ complex (Barry et al., 2015; Corada et al., 2001; Corada et al., 1999; Mehta & Malik, 2006). In as much as the AJ modulates the EC barrier a range of molecules and even cells, ions and solutes may perhaps move across ECs via either a paracellular or transcellular manner (Mehta *et al*., 2014). Increase in permeability of EC barrier is encountered during inflammation and also in vascular pathologies including oedema, tumour angiogenesis as well as sepsis which often results from the disruption of integrity of the VE-cadherin adhesion complex (Aragon-Sanabria et al., 2017; Corada et al., 2001; Crosby et al., 2005; Frye et al., 2015). During inflammation, leukocytes were found to largely pass between the ECs while transmigrating across the endothelial layer (Tsukita *et al*., 2001; Vestweber, 2012; Vestweber *et al*., 2014). Several studies have characterised the various events leading to the breakdown endothelial barrier (Allport et al., 2000; Muller, 2014; Shaw et al., 2001; Tinsley et al., 1999; Vestweber, 2008, 2015). Evelyn and colleagues observed the loss of VE-cadherin from the retracting endothelial junction at the contact region between the ECs and melanoma cells, which led to a further redistribution of VE-cadherin (Voura *et al*., 1998). Furthermore, recently Virginia and colleagues observed activation of SRC, a non-receptor tyrosine kinase, to mediate the disassembly of VE-cadherin in ECs during the process of TEM of metastatic melanoma cells (Aragon-Sanabria *et al*., 2017).

Taken together, these results suggest that ECs VE-cadherin plays an important role during the TEM events in cancers and thus adequate understanding of signalling events modulating the VE-cadherin at the endothelial junctions might identify novel therapeutic targets towards treatment of cancer metastasis.

(VE)-cadherin also interacts with nectins, a cell-cell adhesion molecule, thereby forming a junctional complex, through which they likely mediate the formation of a mature AJ (Noda *et al*., 2010; Wallez & Huber, 2008). The nectin cell adhesion molecules are an immunoglobulin family with four members (nectins 1-4), together with five of the nectin like molecules (Necl 1-5) (Takai *et al*., 2003). Two of the nectin members, nectin 2 and nectin 3 are found to be expressed in endothelial cells (Lopez *et al*., 2001; Takai *et al*., 2003). Like cadherins, nectin interaction leads to the formation of cis and trans dimers across the cell junctions (Takahashi *et al*., 1999). The nectins interact either homophilically and heterophilically via a calcium-independent manner to establish proper cell-cell adhesion (Dong *et al*., 2006; Takai *et al*., 2003). The C-terminus of nectins, bears the postsynaptic density protein-95/ discs large/ ZO-1 (PDZ) binding motif, through which it binds to an actin-binding filament afadin which gets anchored to the actin cytoskeleton of the AJ as well as the TJ (Takahashi *et al*., 1999; Takai *et*

al., 2003). Thus nectin-afadin interaction is crucial for the establishment of strong adherens junctions (Sato *et al*., 2006).

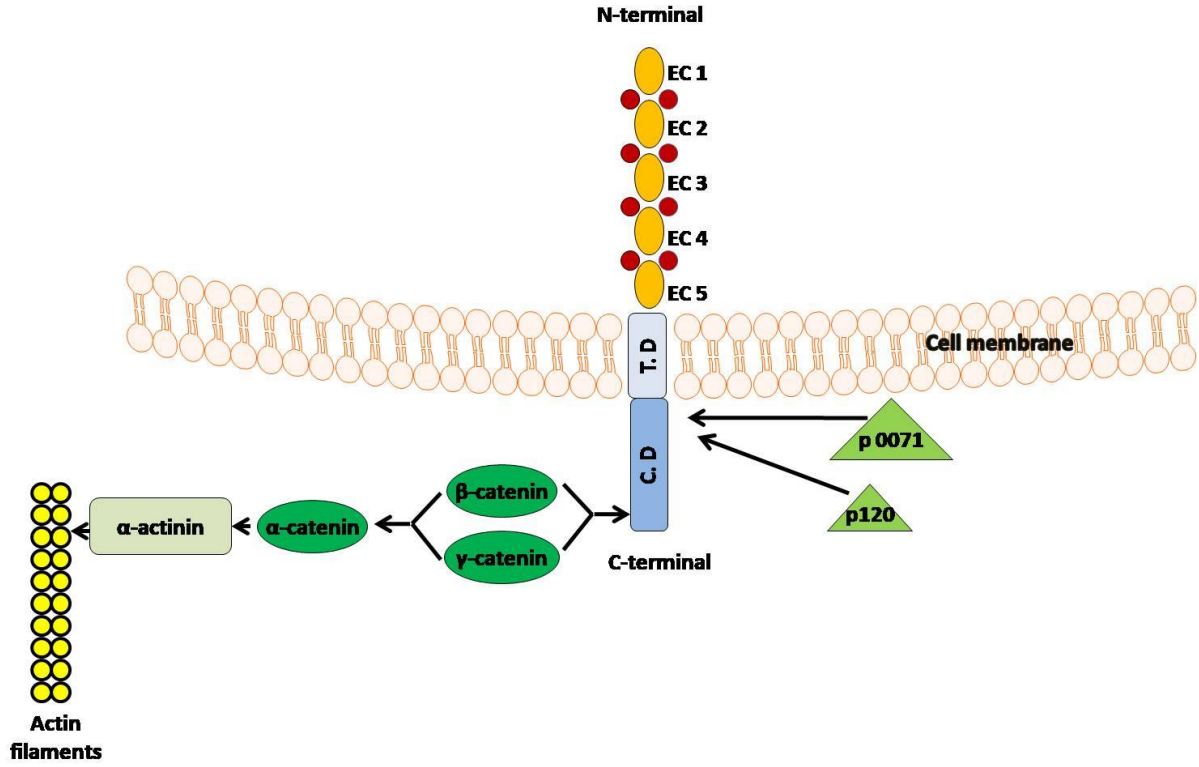

Figure 3. Structure of classical cadherin. The cartoon illustrates the structure of a classical cadherin depicting all the three domains. 5 extracellular domains (EC1-5) (pink) form the extracellular region, with the N-terminus. The EC region expresses two calcium-binding sites. The transmembrane domain (T.D) (light blue).The cytoplasmic domain (C.D, blue) which comprises of two parts thus juxtamembrane domain which serves a region for the binding of armadillo proteins p120 and p0071 bind (light green), and a COOH-terminal domain where either β-catenin or γ-catenin bind (green). α-actinin (green) can bind to either β-catenin or γ-catenin, which can then bind to α-actinin (gray). Finally, α-actinin can bind to the actin cytoskeleton (yellow) to establish the cadherin / catenin complex.

CONCLUSION

It is highly important to note that cancer metastasis is a highly complex event involving both cancer cells and the endothelial junctional complexes. Adequate and proper knowledge of the interactions as well as the role individual role played by these junctions could serve as a target point for the treatment of metastasis which often times is the cause of high mortality. Recently pharmacological agents in form of small molecules which modulates the integrity and function of endothelial junctions are being developed. Further exploration of the role of these junctions will eventually have a great impact towards the management of metastasis in numerous cancers.

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