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Review

The functional roles of T-cadherin in mammalian biology

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Abstract: T-cadherin is a cadherin and cell adhesion molecule that is anchored to the cell surface membrane through a glycosylphosphatidylinositol moiety. T-cadherin lacks a transmembrane and cytoskeletal domain, suggesting that it must interact with other membrane-bound molecules to elicit cellular signaling to modulate normal cellular functions, and alternatively its absence can be a factor in promoting neoplasia. Moreover, apart from binding to itself it can sequester adiponectin to the cell surface. Consistent with these observations, recent research has expanded the scope of T-cadherin's role in cancer, neuronal function, metabolism and cardiovascular disease. In this context, we highlight the experimental and genomic evidence that links T-cadherin with these diseases. In particular, we discuss how T-cadherin homophilic and heterophilic interactions impact on signaling pathways and cellular behavior.

Keywords: T-cadherin; CDH13; adiponectin; metabolism; cancer

1. Introduction

Cadherins are a large class of glycoproteins involved in calcium-dependent homophilic intercellular adhesion. Classical cadherins of subfamilies I and II are often co-expressed with a variant non-classical

cadherin family member, known as Truncated-cadherin (T-cadherin; also known as Cadherin 13: *CDH13* the human homologue; or H (heart)-cadherin), for which a single gene is present in each vertebrate genome. T-cadherin is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor that localizes to caveolin-rich microdomains known as lipid rafts. It is a unique cadherin; while it possesses the general extracellular structure of a classical type I cadherin, and it lacks transmembrane and cytoplasmic domains [1].

T-cadherin's plays a number of diverse roles. Several studies have shown that it can act as a signaling receptor, participate in recognition of the environment, and regulate cell motility, proliferation and phenotype [2,3]. The modulation of cell adhesion by T-cadherin is involved in diverse biological processes such as the negative guidance of the motor axons, hindlimb trajectory during embryonic development [4], regulation of cell growth, migration [5,6] and vascular remodeling in tumors [7]. It is nevertheless less adhesive than classical cadherins and in correlation with its role in cell growth and migration, it may likely play a role in reversible and dynamic cell-cell adhesion and de-adhesion events [8]. Additionally, T-cadherin is a receptor for the adipocytokine adiponectin (APN), indicating that it can modulate metabolism; its function here is only beginning to be elucidated [6]. Herein, we review the literature concerning T-cadherin gene and protein structure, expression pattern, signaling, involvement in neurological process, cancer, and function in metabolism and cardiovascular disease.

2. Gene structure and regulation

CDH13 is expressed on human chromosome 16q24 [9,10], the same chromosomal location as vascular endothelial-cadherin (VE), epithelial-cadherin (E), placental-cadherin (P), CDH8 and CDH11, indicating that these cadherins may be genetically linked, and share similar transcriptional regulation during development and diseases [11]. The T-cadherin gene comprises 1,169,627 base pairs (bps) corresponding to 14 exons which generate a transcript of 3711 nucleotides that in turn produces a cDNA of 2142 bps and a translated protein of 713 amino acids. The protein contains 5 ectodomains, which are each encoded by 2 to 3 exons and a similar structure is observed in E-cadherin and N-cadherin [2,5,12].

Similar to E- and P-cadherin, the T-cadherin promoter lacks a TATA box [12-15]. The T-cadherin gene contains three different predicted positions of transcription start at −73, −120 and −473 bps that have been identified in osteosarcoma cells [15] and lung-cancer cell lines [12]. The proximal 5' flanking region contains a CAAT box at −154 bps and two inverted CAAT boxes at −179 and −450 bps from the translation start site [12,15]. In this light, numerous factors have been shown to regulate T-cadherin gene expression (recently reviewed [5]). For example, the GR (glucocorticoid receptor), progesterone receptor and ER (estradiol receptor) have been confirmed to modulate T-cadherin transcription and/or protein expression [15]. AHR (aryl-hydrocarbon response) elements have also been identified and aryl-hydrocarbon receptor agonists can suppress T-cadherin mRNA and protein expression in vascular smooth-muscle cells [16]. Similarly, the Brain-Specific Homeobox/POU Domain Protein 2 binds to the proximal promoter region of *CDH13* and represses T-cadherin expression in melanoma cells [17]. In vascular smooth muscle cells T-cadherin is downregulated in response to platelet-derived growth factor (PDGF)-BB, epidermal growth factor (EGF) or insulin-like growth factor (IGF) [18]. Taken together, these data show that multiple factors can regulate T-cadherin expression.

3. Protein structure

The amino acid sequence of T-cadherin is well conserved through evolution in all vertebrates [4,5,19]. It consists of 713 amino acids, a pro-peptide, and five cadherin domains (known as EC-1 through -5) common to the classical cadherins [2,4,5]. T-cadherin, like the classical cadherins, requires calcium to mediate homophilic adhesion [20,21]. It shares 30% homology with the classical cadherins [8], but in terms of homophilic binding there are discrete differences. Normally classical cadherins interact on opposing cells through interactions with their N-terminal EC1 domains, leading to the swapping of N-terminal-strands. In contrast, T-cadherin forms X-dimer intermediates through an alternative non-swapped interface near EC1-EC2 calcium-binding sites, creating a clamshell-like structure. Specific mutations within this interface ablate the adhesive capacity of T-cadherin and its ability to mediate neurite outgrowth [19,22]. A recent study has also identified a highly conserved *O-linked* mannose residue in close proximity to EC1-2 contact sites, suggesting that T-cadherin homophilic binding may be regulated at another level [23]. The most striking difference between the classical cadherins and T-cadherin is the absence of transmembrane and cytoplasmic domains. In contrast, T-cadherin is bound to the cell membrane at the carboxy terminus through a GPI anchor [2].

The described molecular weight of T-cadherin varies between 45 to 130 kDa. The reasons for this are diverse. It is feasible that different isoforms or splice variants exist, however only one study has rigorously defined another T-cadherin isoform, T-cadherin 2 in the heart, muscle, liver, skin, somites and neural tissue. Nevertheless, its cDNA only differs from normal T-cadherin cDNA at its 3′ nucleotide sequence where a carboxy-terminal Leu is exchanged to a Lys and extended by five amino acids: Ser, Phe, Pro, Tyr and Val [21]. Additionally, in some cell types, including neurons and smooth muscle cells a partially processed 130 kDa protein precursor is expressed on the cell surface together with the mature T-cadherin protein. The biological role of this unprocessed precursor is not clear, but both forms can bind ligands such as LDL [2,18,24].

Conceivably the strongest reason for the molecular weight changes is glycosylation, while the removal of glycosylation would generate a protein backbone of approximately 72 kDa, suggesting that smaller forms may be due to protein cleavage. There are eight potential glycosylation sites where N-linked oligosaccharides can attach to the protein backbone, that could in turn regulate protein folding and stability. It has been shown that protein molecular weight can differ between tissues and what roles these forms have in regulating T-cadherin activity remains to be determined [5,25].

4. APN binding

Possibly one of the most interesting findings concerning T-cadherin biology is that it can bind the adipocytokine APN. APN is largely secreted from adipocytes and is a multimeric protein that is present in different biologically active isoforms. The basic unit is a 30 kDa monomeric subunit that consist of an N-terminal collagenous domain and globular head. Further post-translational processing generates trimers, trimer-dimers (hexamers), and high molecular weight (HMW) forms of APN. Notably HMW-APN is the dominant form in the plasma, and it is made from multiple trimers into higher order structures ranging from 18–30-mers or longer [26]. A smaller proteolytic cleavage product of APN, known as globular APN (gAPN), has been shown to circulate in human plasma [27]. Significantly, HMW-APN is considered the physiologically most relevant form as it is associated with an improvement in insulin resistance after treatment with thiazolidinedione [28]. In lean healthy

individuals, serum APN concentrations are higher compared to other hormones and growth factors, with a serum concentration of 3–30 μg/mL, or 0.05% of the total serum protein [29].

Three APN receptors have been described. The first two are APN Receptor 1 (AdipoR1), and APN Receptor 2 (AdipoR2) that possess the inverted membrane topology of G-protein-coupled receptors [30]. AdipoR1 binds gAPN with high-affinity and full-length APN (LMW, MMW and HMW-APN forms) with low affinity, whereas AdipoR2 binds both gAPN and full-length APN with moderate affinity. At the signaling level, the binding of APN to AdipoR1/2 increases 5' adenosine monophosphate activated-kinase (AMPK) phosphorylation, to result in the suppression of energy-consuming metabolic pathways such as lipogenesis and gluconeogenesis. A second pathway, stimulated by APN binding to AdipoR2, regulates peroxisome proliferator-activated receptor α (PPARα) ligand activity to increase β oxidation of fatty acids [30,31]. These pathways are activated under conditions of energy stress, and increase catabolic processes to provide cellular energy. The attributes of Adipo-R1 and -R2 signaling and their role in cancer have recently been reviewed [32].

The third APN receptor, T-cadherin, was identified through an exhaustive screen to identify binding partners of HMW-APN [33]. Subsequent publications then showed that in wild-type mice T-cadherin was expressed in vascular cells and APN co-localized to T-cadherin positive cells. In T-cadherin knock-out (KO) mice the association of APN to the vasculature is absent and accumulates in the circulation at 4-fold greater levels. These data suggest that T-cadherin's principle function is to sequester APN to responsive tissues, and this has been shown for the heart, muscle and vasculature. Importantly, in T-cadherin KO mice the cardiac and vascular tissues lacking T-cadherin become non-responsive to APN, while AMPK signaling is inactivated, despite the sustained expression of AdipoR1/R2 [34-36]. The implications of APN and T-cadherin interactions will be further discussed below. Moreover, we will also present published work, showing that T-cadherin in its guise as a cadherin regulates homophilic adhesion, suggesting that decreased T-cadherin expression can be associated with oncogenic transformation in various epithelial tissues.

5. T-cadherin cellular functions

T-cadherin is expressed in different tissues and cell types that includes the nervous system, the cardiovascular system consisting of the endothelium, vascular smooth muscle cells, cardiomyocytes and aorta, skeletal muscle, retina, pancreas, and epithelium such as the skin, prostate, mammary and intestine. Some of the roles of T-cadherin in these tissues have been extensively and recently reviewed elsewhere [5,37]. Here, we will focus on the nervous and the cardiovasculature systems, blood vessels and select tumour types.

5.1. Nervous system

T-cadherin was originally defined in the chick embryonic nervous system during motor neuron extension at early developmental stages [5]. Studies have shown that it is expressed in the murine and rat developing adult brain where it mediates its function through homophilic and heterophilic interactions to regulate cell migration, neuronal outgrowth and axon guidance [2,4,38,39]. Related to this research numerous genome-wide association studies (GWAS) identified T-cadherin genetic variants as risk factors in association with a number of neuronal developmental disorders, including attention deficit/hyperactivity disorder (ADHD), drug and alcohol abuse, and mental health diseases

such autism, schizophrenia and bipolor behavior, and personality traits such as extraversion and violent behavior [25,40-42] (recently reviewed in [37]).

In this manner, the group of Lesch speculated that T-cadherin is important for the guidance of neurons during development and for modulating specific synaptic contacts in the brain [43]. In wild-type mice they found that T-cadherin is expressed in the hippocampus and confined to distinct classes of interneurons. They tested the function of T-cadherin in this cellular compartment by generating whole body KO T-cadherin mice, through breeding *Cdh13* conditional (floxed) mice with constitutive Cre-deleter mice, to generate *Cdh13* knockout (*Cdh13*$^{-/-}$) mice. The absence of T-cadherin promoted increases in basal inhibitory, but not excitatory, synaptic transmission in CA1 pyramidal neurons, to result in deficits in learning and memory. In behavioral studies, male *Cdh13*$^{-/-}$ mice had normal attentional performance, impaired spatial learning in the Barnes maze, and following delay fear conditioning reduced cued fear memory. The authors suggest that modifications in the *Cdh13* gene may be a factor contributing to an excitatory/inhibitory imbalance as observed in neurodevelopmental disorders, such as ADHD and autism.

In the brain T-cadherin is expressed by the ventral tegmental and substantia nigra pars compacta neurons that are involved in reward, locomotion control and the modulation of cognitive functions. Given that GWAS identified T-cadherin variants that are associated with reward phenotypes, studies conducted by Drgonova et al. [44] further extended and rigorously evaluated T-cadherin's role in this behavior. Here they utilized total constitutively altered *Cdh13*$^{-/-}$ and conditional *Cdh13*$^{-/-}$ mice, the later intercossed with mice expressing a Cre recombinase and mutant form of the estrogen receptor fusion protein (Cre/ERT2); under the control of a Ubiquitin C (*UBC*) promoter. In response to cocaine dependency the authors evaluated the behavioral responses of both lines of *Cdh13*$^{-/-}$ mice. Mice with lifelong reductions in T-cadherin had increased place preference and thus reward at 5 mg/kg, but reduced preference at 10 mg/kg. In mice where T-cadherin expression was reduced in adulthood, there was also an increase in preference for places with a modest dose of cocaine, suggesting that alterations in the T-cadherin gene later in life could be connected to this behavior in patients, and alters the dose-response relationship for cocaine reward and reinforcement. In comparison to that reported by Rivero et al. [43] the authors broaden their locomotion studies to include both sexes, and found that total KO *Cdh13*$^{-/-}$ male and not female mice had reduced locomotion. Although not defined by sex, aged KO mice had reduced locomotion. In motor conditioning and learning total KO mice of both sexes showed faster task acquisition and learning in the Rotarod and Morris water maze test. Further, at the molecular level, in depth inspection found reduced dopamine levels and increased immunoreactivity for dopamine transporters in the cerebral cortex, whereas in the other regions of the brain they were unchanged. These data are significant, while the neurotransmitter dopamine is associated with reinforcing the effects of drug abuse, the pathophysiology of ADHD and schizophrenia [45]. This in turn suggests that T-cadherin regulates the activity of the neurons that are involved in these responses and that the modulation of neuronal T-cadherin gene activity could be a novel therapeutic strategy to treat addiction, ADHD and/or schizophrenia.

Recent work has extended the role of T-cadherin outside the brain nervous system. Here the authors considered the molecular character of proprioceptive sensory neurons, which are specialized sensory receptors on nerve endings found in muscles, tendons and joints, and detect delicate changes in movement, position, tension, and force. The elegant paper by Poliak et al. shows that it is the positional character of the limb mesenchyme that determines the molecular identity of *Cdh13* proprioceptive neuron populations, thus suggesting that T-cadherin plays a role in patterning the nervous system [46].

Given T-cadherin's roles in the development of the nervous system, studies have shown that perturbations in T-cadherin function are associated with neuronal oncogenesis. T-cadherin is downregulated in gliomas and when overexpressed in C6 glioma cells reduced cell growth and motility, and increased cell attachment and homophilic adhesion. The overexpression of T-cadherin induced aneuploidy, and was associated with G2 phase growth arrest and the requirement of p21 Cdk interacting protein 1 (CIP1)/wild-type p53 activated factor (WAF1) expression [47]. In sum, in the nervous system, T-cadherin plays an important role during development in the patterning the nervous system, and variants or reduced T-cadherin expression are associated with neurological disorders and cancer.

5.2. The cardiovascular system, angiogenesis and metabolic control

T-cadherin is expressed in cardiomyocytes, endothelial and smooth muscle cells, and myofibers in select organs, such as the heart, lungs, liver and skeletal muscle [48,49]. A large body of work pertaining to *in vitro* and *in vivo* models has in part elucidated the function of T-cadherin in these systems. A recent review by Philippova et al. [5] extensively covered this work and we will only consider reports post this publication.

As T-cadherin is extensively expressed in the heart, studies were pursued by Denzel et al. with wild-type, T-cadherin KO and double KO T-cadherin and APN mice, subjected to either hypertrophy or ischemic reperfusion [35]. In wild-type mice there was extensive co-localization of T-cadherin and APN to the cardiomyocytes *in vivo*. In contrast, in T-cadherin KO mice APN did not associate with cardiac tissue, and APN levels were increased in the serum. As expected, treated T-cadherin KO mice had enhanced cardiac hypertrophy, similar to that observed in APN KO mice. Importantly, T-cadherin was necessary to induce the phosphorylation of AMPK a target of APN signaling.

In an associated story, mice were subjected to a model of hind limb ischemia where blood flow was surgically disrupted in one limb [36]. Similar to that in cardiac stress models, the T-cadherin KO and APN KO mice had a similar response with a reduced blood flow compared to wild-type controls. The application of APN by an adenovirus rescued the impaired revascularization phenotype in APN KO mice but not in T-cadherin-null mice. In cellular models, T-cadherin knock-down by siRNA in endothelial cells ablated the ability of APN to promote cellular migration and mitosis. These data suggest that in both instances APN binding to T-cadherin protects from stress-induced pathological cardiac remodeling and is essential for mediating the vascular actions of APN.

An important feature of the above models is that APN is not associated with the cellular membranes and accumulates in the serum of T-cadherin null mice, whereas in the wild-type condition APN is bound to T-cadherin positive membranes and circulates at normal serum levels. In APN KO mice, the levels of T-cadherin protein are reduced and together the data suggests the expression levels of APN and T-cadherin are interrelated [35]. To test this relationship Matsuda et al. [48] used adenoviral APN overexpression constructs and found that increased APN raised T-cadherin protein levels *in vivo* and *in vitro*. Moreover, they observed in wild-type and T-cadherin KO mice similar levels of APN secretion from adipocytes, the major source of serum APN. In addition, they found that T-cadherin was required to bind APN to the surfaces of cells *in vitro* and *in vivo*, while T-cadherin KO mice and cells treated with T-cadherin siRNA had diminished binding of APN. Previously, it had been shown that T-cadherin can be enzymatically cleaved from the cell surface with phosphatidylinositol-specific phospholipase (PI-PLC) C [20]. The authors tested the relevance of PI-PLC *in vivo* and found that the injection of PI-PLC into wild-type mice increased serum APN concentrations and reduced

tissue levels of APN in the heart and skeletal muscle. Interestingly, they found *in vitro*, that the pretreatment of endothelial cells with serum containing APN reduced the action of PI-PLC mediated T-cadherin cleavage. *In vivo*, the GPI phospholipase D (GPI-PLD) is the endogenous cleavage enzyme for GPI-anchored proteins. Here the authors found elevated levels of GPI-PLD in APN KO mice and the *in vivo* administration of adenovirus producing APN reduced the plasma levels of GPI phospholipase D. In summary, this report showed that both circulating and tissue-bound APN levels depend on T-cadherin and, in turn, regulate tissue T-cadherin levels through a positive feedback loop that suppresses phospholipase-mediated T-cadherin release from cells [48].

T-cadherin is also expressed on endothelial cells and is up-regulated on the tumor vasculature of murine clinical tumor models of breast, melanoma, lung cancer and rhabdomyosarcoma [7]. The role of APN and T-cadherin interactions in neoangiogenesis is to support endothelial migration and mitosis [36]. However, an alternative model has also been pursued by the Resink group where T-cadherin regulates vascular functions independently of APN. Research from this group has characterized a number of T-cadherin's novel functions and interacting partner molecules that are important in cellular signaling [5].

In initial experiments, Joshi et al. found that T-cadherin endothelial expression increases during conditions of oxidative stress that coincides with endothelial cell migration, proliferation and apoptosis/survival [50]. The overexpression of T-cadherin in endothelial cells and subsequent induction of cellular stress (serum-deprivation, TNF-alpha, actinomycin D, staurosporine) reduced apoptosis with increased cell survival. The application of the PI3K-inhibitor wortmannin or the mTOR-inhibitor rapamycin increased cell death, suggesting that T-cadherin overexpression protects against stress-induced apoptosis through activation of the PI3K/Akt/mTOR survival signaling pathway [50]. Studies pursued by the same author revealed that T-cadherin-overexpressing endothelial cells had increased levels and nuclear accumulation of transcriptionally active β-catenin. The expression of constitutively active glycogen synthase kinase (GSK3β) reduced the action of T-cadherin on active β-catenin accumulation, and limited proliferation, and survival. Significantly, it was observed that Integrin-linked kinase (ILK), a regulator of Akt and GSK3β, associated with T-cadherin in immunoprecipitation experiments and via microscopy. Further, the application of ILK-siRNA eliminated the role of T-cadherin in promoting Akt and GSK3β phosphorylation, active β-catenin accumulation, and survival. In sum, these studies show a model through which GPI-anchored T-cadherin can via ILK regulate β-catenin activity and vascular endothelial cell survival [51].

Through immunoprecipitation experiments of lysates from endothelial cells and subsequent high-performance liquid chromatography (HPLC) they found associations between T-cadherin and glucose-related protein GRP78, and separately with integrin β3. The cell surface association of Grp78 and integrin β3 with T-cadherin was established by surface biotinylation and immunoprecipitation, and confocal microscopy. Grp78 is a HSP70 molecular chaperone that binds newly synthesized proteins and maintains them in a state competent for subsequent folding and oligomerization; it participates in pro-survival responses to ER stress. Therefore, the investigators sought out the roles of Grp78 and T-cadherin in endothelial cells. Functionally, the application of anti-Grp78 blocking antibodies and Grp78 siRNA to T-cadherin expressing endothelial cells reduced the activity of the Akt/GSKβ signaling and survival [52].

Further studies tested the role of T-cadherin in unfolded protein response (UPR) signaling during ER stress. Using a variety of ER stress-inducing compounds it was observed that T-cadherin mRNA and protein levels increased. The subsequent overexpression or silencing of T-cadherin in the endothelial cells respectively decreased or increased the ER stress-induced increase in Grp78,

phospho-eIF2α (phosphorylated eukaryotic initiation factor 2α) and CHOP (C/EBP homologous protein) and active caspases [53]. In a separate study Nakamura et al. utilized human retinal microvascular endothelial cells (HRMEC) and a mouse model of oxygen-induced retinopathy (OIR). They observed that after the induction of ER stress *in vitro* and *in vivo* Grp78 gene and protein expression was upregulated and this coincided with increased HRMEC proliferation and migration. Moreover, on the induction of stress, Grp78 increasingly interacted with T-cadherin. Grp78 was expressed in the pathological vasculature and retinal microvascular endothelial cells, which have been previously shown to express T-cadherin, and T-cadherin absence is associated with reduced retinal angiogenesis [34]. In sum, these reports suggest that in endothelial cells T-cadherin protects against ER induced stress and apoptosis and promotes endothelial cell proliferation and migration.

Earlier work by the Resink group identified T-cadherin as a receptor for low density lipoprotein (LDL) [24,54,55] and separately it was found that LDL-T-cadherin interactions were important in mediating vascular cell proliferation [56]. In line with a role in human disease where aberrant smooth muscle cell proliferation is a factor in atherosclerosis, restenosis and tumour angiogenesis, the Resink group found that T-cadherin overexpression increased cellular detachment, migratory responses, proliferation and angiogenesis [57-59]. Recent *in vitro* work has extended their concepts where they found that T-cadherin overexpression promoted insulin insensitivity while insulin had a reduced ability to stimulate the Akt/mTOR signaling [60].

However, impacting on these concepts is that APN is a known insulin sensitizer [32], and upregulated T-cadherin should correspond with enhanced APN binding. A recent examination of APN and T-cadherin in clinical specimens of aortic and atherosclerotic lesions, and periadventitial adipose, observed that APN was present on adipocytes and T-cadherin expression was increased and stained vascular smooth muscle and endothelial cells [61]. Moreover, a recent study by Fujishima et al. employing murine models of atherosclerosis showed that APN and T-cadherin interactions were protective against both neointima formation and atherosclerosis [62]. Intriguingly, Phillippova et al. separately found that T-cadherin was present on endothelial microparticles, elevated in the serum of atherosclerotic patients, and through homophilic interactions to facilitate angiogenesis [63]. However, in light of the work published by Matsuda et al. [48] and Fujishima et al. [62] it is also a plausible that the feedback loop regulating APN and T-cadherin levels could become dysregulated and result in the release of T-cadherin from cardiac and blood vessel cell surfaces, which would in turn limit the cardio-protective response of APN.

Taken together, given that APN and T-cadherin have been shown to be critical in murine models of cardiac function, angiogenesis and atherosclerosis, these data suggest that APN and T-cadherin interactions are probably a stronger contributor in these cellular compartments than just T-cadherin homophilic interactions alone. In this light, an investigation of APN and T-cadherin interactions in the realm of the described interactive partners and signaling, and the potential aberrant cleavage of T-cadherin from cell surfaces is required

An interesting aspect of T-cadherin is that it has been found in pancreatic β-cells and is associated with insulin granules in both murine and human β-cells [64]. Functional analyses showed that T-cadherin was required for insulin release *in vitro* and *in vivo.* In particular, T-cadherin KO primary islets were deficient in glucose-induced but not KCl-mediated insulin secretion, acute first phase insulin release was unaltered, and second phase insulin release was impaired. Moreover, the KO mice showed progressive glucose intolerance by five months of age without concomitant changes in peripheral insulin sensitivity, suggesting that pancreatic control was responsible for the phenotype.

Interestingly, APN was not associated with T-cadherin in the β-cell granules. Together this report illustrates a novel attribute of T-cadherin function in regulating insulin secretion and metabolism independent of its known APN interactions. It remains to be determined whether T-cadherin down-regulation or mis-function in β-cells has a role in human disease.

5.3. T-cadherin and cancer

CDH13 is located to chromosomal region 16q24, a region that is often subject to oncogenic modifications in several cancers. Furthermore, the *CDH13* gene has been implicated in different types of human cancers, where *CDH13* gene expression has been silenced by methylation. Similarly, with regards to its described function in smooth muscle and endothelial cells T-cadherin can regulate cell proliferation and motility. As such, in cancer cell lines increased T-cadherin expression correlates with increased adhesion and reduced proliferation, and its absence due to methylation and corresponding limited expression is associated with enhanced tumor cell aggressiveness and proliferation [65]. This had led to T-cadherin being described as a tumor suppressor in numerous cancer types and possibly a biomarker for certain cancers [66].

Breast cancer: T-cadherin expression is reduced in human breast carcinoma cell lines and breast cancer specimens. The re-introduction of T-cadherin reduced tumor cell growth and invasive tumor cell morphology [67]. Follow-up studies showed that *CDH13* was rarely mutated in human breast cancer, but the promoter was methylated [68,69], and its reduced expression as part of a group of other factors is an indicator as a marker of aggressive invasion in both estrogen receptor-positive and -negative breast tumors [70]; negative expression correlates with a poor prognosis in patients with axillary lymph node-positive breast cancer [66]. In a mouse model of breast cancer using the mammary tumor virus-polyoma middle T-antigen (MMTV-PyMT) model crossed into T-cadherin null mice, breast tumor growth was delayed, and tumors showed reduced blood vessel density, and increased tumor hypoxia with aggressive pathology, as illustrated by metastasis to the lungs [34]. Significantly, histology showed that APN was absent from the T-cadherin KO tumor blood vessels and in the wild-type condition APN was associated with the vasculature and no staining was present on the epithelial ducts. Thus, these data suggest that APN and T-cadherin interactions are important for regulating breast tumor angiogenesis. Whether the loss of epithelial T-cadherin is the sole limiting factor for increasing metastasis *in vivo* remains to be determined. Furthermore, the human analyses described above have been derived independent of T-cadherin's association with APN, thus it is an imperative to determine whether low serum APN and breast T-cadherin levels are a risk for aggressive breast cancer.

Digestive tract cancers: In cancers of the digestive tract, T-cadherin loss has been suggested to be associated with colon cancer [71] and gastric cancer [72-74]. Recent data has also shown that *CDH13* gene methylation occurs in colon cancer cell lines, and in the non-neoplastic mucosa as a predictor of ulcerative colitis associated colorectal cancer (CRC) [75,76], suggesting that T-cadherin loss is an early feature of CRC.

Lung cancer: A number of studies have suggested an association between T-cadherin and lung cancer. Sato et al. [12] initially observed that T-cadherin was inactivated in lung cancer cell lines and human tumors. Subsequent studies showed that *CDH13* loss and aberrant methylation was associated with increased tumorigenicity and progression in human non-small cell lung cancer (NSCLC) [77]. *CDH13* promoter methylation in conjunction with cyclin-dependent kinase inhibitor 2A gene p16, the Ras association domain family 1 gene RASSF1A, and adenomatous polyposis coli gene APC,

predicted early NSCLC recurrence after surgery [78]. Thus, in NSCLC, *CDH13* promoter methylation assists in identifying patients with cancer relapse and predicts patient survival.

Skin cancer: Initial studies identified that T-cadherin was expressed in the basal layers of murine and human skin [21], and suggested a possible role in epithelial barrier maintenance [79]. Further studies by Takeuchi et al. found that T-cadherin was absent in cutaneous squamous cell carcinoma (SCC) [80] and can negatively regulate SCC proliferation [81], furthering an important role for T-cadherin in regulating keratinocyte proliferation and skin tumorigenesis. Mechanistically, research into T-cadherin's role in skin cancer has revealed signaling partners by which T-cadherin can mediate its effects on cell behavior. In SCC T-cadherin expression regulated the surface levels of β1 integrin, which can bind to the extracellular matrix (ECM). In cells expressing limited T-cadherin, β1 integrin was internalized in caveolae and T-cadherin expression suppressed β1 integrin internalization. Moreover, the authors found in T-cadherin overexpressing SCC cells reduced EGF receptor (EGFR)-phosphorylation. In addition, the application of EGF and EGFR inhibitors illustrated that EGFR activation stimulated β1 integrin internalization. These *in vitro* studies show that T-cadherin can modulate the invasive ability of SCC by regulating surface levels of β1 integrin [82].

Separate work by Pfaff et al. found that the ablation of T-cadherin in A431 cells, a model of SCC, induced a disorganized cell phenotype, and increased cell motility and invasion. In contrast, the overexpression of T-cadherin markedly reduced cell invasion [83]. In human SCC they observed that T-cadherin loss was associated with histological features of a more malignant and invasive phenotype of SCC. Further studies by the same group then evaluated the effect of T-cadherin on SCC growth *in vivo*. They ablated or overexpressed T-cadherin in A431 cells and found that both increased tumor growth. They found contrasting roles, where the ablation of T-cadherin, increased tumor cell proliferation *in vitro* and *in vivo*, and increased Erk1/2:p38MAPK activity after EGF treatment; and T-cadherin overexpression reduced tumor cell apoptosis, and increased tumor angiogenesis and lymphangiogenesis to promote tumor growth [3]. Furthermore, they explored T-cadherin's effect on EGFR signaling and found that the loss of T-cadherin released EGFR from lipid rafts to enhance ligand-dependent EGFR activation, whereas in contrast T-cadherin overexpression promoted the retention of EGFR to lipid rafts and limited EGFR activation [84]. An analysis of T-cadherin's role in SCC metastasis illustrated that limited T-cadherin increased the metastatic potential and aggressiveness of SCC A431 cells, largely through promoting arrest and extravasation through the vascular wall and facilitating the potential of metastases to grow at new sites [85]. Together these data suggest that the loss of T-cadherin is a promoter of aggressive SCC and possibly an avenue for therapeutic intervention.

Prostate cancer: A study found in castrated mice followed by hormone replacement, that T-cadherin was upregulated on testosterone treatment. Androgen response elements were found in the *CDH13* promoter and *CDH13* gene expression was often reduced in a majority of human prostate cancer cell lines. Furthermore, T-cadherin expression limited, and the knock-down of T-cadherin in BPH1 cells promoted tumorigenesis, respectively [86]. In contrast, a human based study showed that increased levels of T-cadherin protein are associated with prostate carcinoma in organ-confined tumors, when compared to benign disease [87]. Thomas et al. through a GWAS found a single nucleotide polymorphism (SNP) located in intron 1 of T-cadherin is associated with aggressive prostate cancer [88]. What its function is on tumor progression remains to be determined. Thus, at different levels T-cadherin has been associated with prostate cancer.

To address the functional roles of T-cadherin in prostate cancer studies conducted by Maslova et al. investigated the role of T-cadherin in prostate cancer cell phenotype [89]. Here it was found that EGFR and

the insulin growth factor receptor (IGF-IR) can wield contrasting effects on cancer cell phenotype, and these effects could be modulated by T-cadherin. The investigators used two prostate cancer cell lines, malignant (DU145) and benign (BPH-1) for their studies. T-cadherin overexpression in both cell lines promoted invasion and migration, and T-cadherin silencing in BPH-1 cells limited migration and invasion. Furthermore, it was found that increased T-cadherin expression levels in both cell types reduced EGFR and IGF-IR activity, whereas conversely T-cadherin silencing increased EGFR and IGF-IR receptor phosphorylation. Moreover, EGFR and IGF-IR have contrasting effects on cell phenotype. EGFR activation with EGF promoted dissemination, invasion, and polarity loss, and the application of NVP-AEW541, an IGF-1R inhibitor, decreased aggressive cell behavior. Conversely, the inhibition of EGFR with gefitinib promoted a contained epithelial phenotype, and the subsequent application of NVP-AEW541 induced aggressive characteristics. IGF-1R activation with IGF-1 rescued epithelial morphology and decreased invasion. In sum, prostate cancer cell morphology and aggressiveness depends on the balance of activity between EGFR and IGF-1R, and this is in turn modulated by T-cadherin.

Bladder cancer: Studies with human tissues has linked *CDH13* gene downregulation and methylation with bladder transitional cell carcinoma [90-92], and as a potential biomarker in non-muscle invasive bladder cancer [93]. In an additional study T-cadherin expression in bladder transitional cell carcinoma (TCC) tissues was significantly decreased when compared to normal bladder epithelial tissues. Significantly, T-cadherin expression in the muscle-invasive group was decreased when compared against the non-muscle-invasive group. It was also observed that matrix metalloproteinase (MMP2) expression was increased in bladder TCC, in the main in muscle-invasive tumors. The knock-down of T-cadherin into invasive 5637 cells, reduced cellular migration, invasion and adhesion, and promoted MMP2 expression [94].

Other cancers: Clinical studies have also linked reduced T-cadherin expression to chronic myeloid leukemia [95] and pancreatic cancer [96], however functional studies have not as yet been performed to validate its role in these cancers.

Overall and of relevance to the studies discussed, decreased T-cadherin expression is linked with breast, skin, lung, skin and bladder cancers, and increased T-cadherin expression is associated with prostate cancer (Table 1). Given that APN can positively regulate T-cadherin protein levels [48], it will be important to determine if reduced levels of APN as observed in overweight and obese patients, correlates with reduced epithelial T-cadherin expression and increased cancer risk.

Table 1. Illustrates the association of T-cadherin with human cancers, and the elucidated signal transduction targets.

Cancer Type	T-cadherin gene expression alteration	Signal transduction targets	Supporting publications
Breast	Downregulated	N.D.	[34,67]
Digestive	Downregulated	N.D.	[71-74]
Lung	Downregulated	N.D.	[12]
Skin	Downregulated	β1 integrin, EGFR	[3,80-83]
Prostate	Protein upregulated, SNP found	EGFR, IGF-IR	[86-89]
Bladder	Downregulated	N.D.	[91-93]

N.D. = not done

6. Links with human metabolic disease

Given the important roles of T-cadherin in oncogenesis, neuronal function, vascular and cardiac health, alterations in the T-cadherin gene would have implications for homophilic interactions and APN binding, that could then impinge on increased risks for specific human diseases. Alterations in *CDH13* gene sequence in the neuronal compartment and their relationship to behavioral change have already been presented. Thus, the focus here will be to consider GWAS that have identified a correlation between T-cadherin and metabolic disease.

Knowing that a positive feedback regulation exists between APN and T-cadherin, and in turn impacts on APN levels in the tissues and serum [48], a regressive change in the *CDH13* gene promoter or coding sequence could reduce T-cadherin protein levels, and ultimately the direction of APN to specific tissues and the provision of its protective signaling functions. The identification of *CDH13* SNPs could also allow the identification of at-risk patients and corresponding therapeutic intervention or lifestyle changes. In this light, a number of GWASs have been presented showing an association between *CDH13* SNPs and APN levels. For example, two Korean studies have showed in specific population groups an association of the *CDH13* SNP rs3865188 with higher APN levels [97]; and confirmed that individuals containing the T allele (mutant form) of rs3865188 had significantly lower and insulin levels, and significantly higher plasma triglyceride than those with the A allele (wild-type) [98]. Additionally, a GWAS on Filipino women identified the mutant SNP rs3865188 to be associated with lower APN levels [99]. Furthermore, a separate French study identified a relationship for two *CDH13* SNPs. The rs11646213 A allele was significantly associated with type 2 diabetes (T2D) as represented by an increased body mass index (BMI) and glycated hemoglobin (HbA1c), and decreased plasma levels, whereas the rs3865188 (A allele) variant, was associated with a lower risk for T2D, lower BMI and HbA1c, and fatty liver as an indicator of hepatic metabolic disease, and higher plasma levels [100]. In other population groups different *CDH13* SNPs have been revealed e.g. Ling et al. examined a West European cohort and showed that the SNP rs7195409 (intron 7 of *CDH13)*, is associated with lower APN levels [99].

Apart from relating *CDH13* polymorphisms to reduced serum APN levels, studies have also connected *CDH13* SNPs to other features of the metabolic syndrome. A European study incorporating two German and Estonian cohorts located the *CDH13* SNP rs11646213 (17.9 kb upstream of the *CDH13* gene) in individuals with increased blood pressure [10]. A Taiwanese group found that the *CDH13* GG genotype of SNP (rs4783244) located in intron 1 of the *CDH13* was associated with an increased waist circumference, a low APN level, a high level of triglyceride and fasting glucose, and an increased risk of the metabolic syndrome and T2D [101]. However, there are confounding results, as recent data from a Mexican group illustrated that the T > A (rs11646213) gene polymorphism of *CDH13* is associated with a decreased risk of developing hypertension in Mexican population cohort. This counters that seen by other investigators who have observed that the A allele of *CDH13* T > A (rs11646213) is associated with higher triglycerides levels, systolic blood pressure, and lower high-density lipoproteins in Caucasian patients with metabolic syndrome [102]. Such discrepancies could be due to the allelic distribution of specific *CDH13* polymorphisms or variances with the ethnic origin. This could also mean that any future therapeutic targeting increased *CDH13* gene transcription may have diverse effects in different population groups.

Finally, a recent study has merged T-cadherin's epithelial expression in the colon and metabolic functions with serum levels of APN and an increased risk of colorectal cancer (CRC). Here, the

investigators identified *CDH13* polymorphisms *rs3865188* and *rs3774261* that were associated with lower plasma APN levels and increased CRC risk [103]. Thus, in sum genomic studies have revealed strong links between T-cadherin expression and APN levels with the metabolic syndrome, fatty liver disease and cancer.

7. Conclusion and Perspectives

Taken together T-cadherin has been ascribed diverse roles as a regulator of neuronal function, endothelial and smooth muscle cell activity, and is involved in epithelial oncogenic transformation and as a regulator of metabolism through its binding to APN and insulin granules (Table 2). At the molecular level, it has been linked with to a number of signal transduction mechanisms, through which it can mediate normal tissues homeostasis and alternatively when absent, be one of the events that promotes aberrant cellular behavior and ultimately human disease (Figure 1). In this light and importantly, genomic studies have linked reductions in *CDH13* expression or alterations in the sequence of the *CDH13* gene to human diseases associated with neurological disorder, metabolism and oncogenesis.

Table 2. Lists the principle publications concerning T-cadherin homophilic and adiponectin and T-cadherin interactions in mediating potential human diseases.

Cellular functions	Homophilic T-cadherin interactions	Adiponectin and T-cadherin interactions	Disease state/model
Neuronal	[2,38,44]	N.D.	ADHD, drug and alcohol abuse, autism, schizophrenia, bipolar, extraversion, violent behavior, sensory neurons
Cardiovascular	[104]	[35,62]	Hypertrophy, ischemia, atherosclerosis, restenosis
Angiogenic	[59]	[34,36]	Limb ischemia, tumor blood vessel growth
Cancer	[67,79,84,89]	[32]	Breast, prostate, skin, glioma
Metabolic	[10,99,101,102]	[64]	Diabetes, insulin release, fatty liver, metabolic syndrome

N.D. = not done

Thus, the question has to be posed, is a strategy to increase T-cadherin expression or correct its sequence a plausible therapeutic strategy to treat human disease? Such an approach to increase T-cadherin expression will be required to target T-cadherin promoter sequences by gene editing, or the transcriptional machinery that regulates *CDH13* transcription, or factors that increase *CDH13* promoter methylation. This will in turn require exquisite specificity to distinct neurons or tumor cells to alter disease course and progression. However, given that in APN KO mice the levels of T-cadherin protein are reduced and serum APN levels fall in line with increased adiposity, could a strategy of increasing T-cadherin levels be through weight loss or by therapeutically promoting increases in serum

APN levels? Will increases in serum APN increase T-cadherin expression in other cellular compartments, such as neurons, that could in turn alleviate disease?

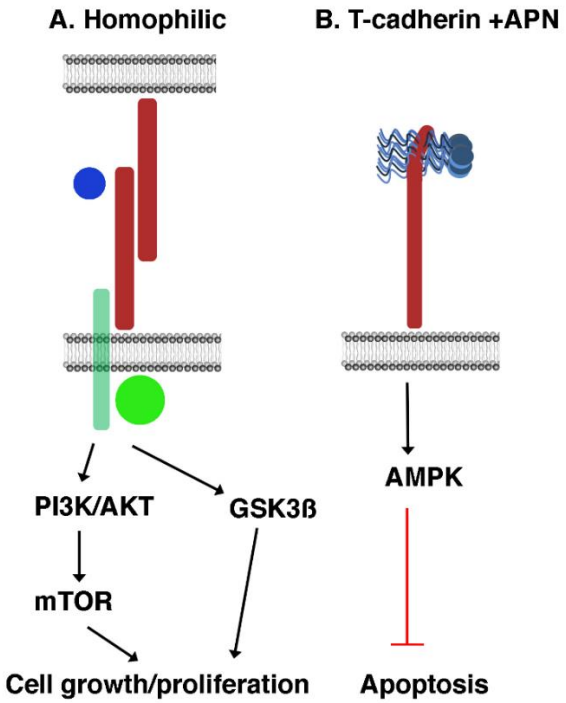

Figure 1. Overview of T-cadherin mediated signaling. A, T-cadherin can interact with itself and via transmembrane molecules such as the insulin receptor, β1 integrin and Grp78, (light green bar) to modulate ILK and EGFR (green ball) activity. Downstream it can in turn regulate PI3K/AKT/mTOR and GSK3β activity and cell growth and proliferation. B, T-cadherin and APN interactions lead to the phosphorylation of AMPK and the inhibition of apoptosis. Note: T-cadherin can also interact with LDL (blue ball) to mediate cell proliferation.

Alternatively, there are also instances where T-cadherin expression increases in the smooth muscle cells of diseased hearts and is expressed by the tumor vasculature. It is plausible then that T-cadherin on the vasculature could be a direct target, but off-target effects to other T-cadherin positive organs could be a potential cofounder. In the instance of the heart, any reduction in T-cadherin levels could limit the protective role of APN. Therefore, at this stage given the numerous functions and diverse tissue expression of T-cadherin it is too early to call whether a T-cadherin therapeutic strategy will be successful. However, with the advent and development of more specific gene targeting and cell specific strategies this may change.

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Conflict of interest

The authors have nothing to disclose.

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