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New Functions for Alpha-Catenins in Health and Disease: From Cancer to Heart Regeneration

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Abstract

Strong cell-cell adhesion mediated by adherens junctions is dependent on anchoring the transmembrane cadherin molecule to the underlying actin cytoskeleton. To do this, cadherin cytoplasmic domain interacts with catenin proteins, which include α-catenin that binds directly to filamentous actin. Originally thought to be a static structure, the connection between the cadherin/catenin adhesion complex and the actin cytoskeleton is now considered to be dynamic and responsive to both intercellular and intracellular signals. Alpha-catenins are mechanosensing proteins that undergo conformational change in response to cytoskeletal tension thus modifying the linkage between the cadherin and the actin cytoskeleton. There are three α-catenin isoforms expressed in mouse and human: αE-catenin (CTNNA1), αN-catenin (CTNNA2), and αT-catenin (CTNNA3). This review summarizes recent progress in understanding the *in vivo* function(s) of α-catenins in tissue morphogenesis, homeostasis, and disease. The role of α-catenin in the regulation of cellular proliferation will be discussed in the context of cancer and regeneration.

Keywords

α-catenin; adherens junction; mouse models; hyperproliferation; arrhythmogenic cardiomyopathy

Introduction

Alpha-catenins are mechanosensing proteins associated with the cytoplasmic domain of classical cadherins, a family of transmembrane cell adhesion molecules, found in adherens junctions (AJs) of well-polarized cells (e.g., epithelial cells). Three α-catenin subtypes are present in mouse and human: CTNNA1 (αE-catenin, epithelial), CTNNA2 (αN-catenin, neural), and CTNNA3 (αT-catenin, testis). Alpha-catenins contain three vinculin homology domains, N-terminal α-catenin-binding site, and a C-terminal domain that interacts with F-actin facilitating linkage of the cadherin/α-catenin complex with the actin cytoskeleton (Kobielak and Fuchs, 2004). The α-catenin homolog, plakoglobin (α-catenin), is also capable of binding the C-terminus of cadherins and interacting with α-catenins. In addition

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Conflict of interest

None

to vinculin, α-catenins can interact with myriad actin-binding proteins either directly or indirectly thus regulating actin dynamics and assembly at the AJ (Fig. 1). Recent studies are beginning to elucidate the molecular mechanism(s) by which α-catenins and its actin-binding partners, particularly vinculin, transduce mechanical force from the cadherin/catenin complex to the cytoskeleton (Barry et al., 2014; Leerberg et al., 2014; Thomas et al., 2013; Yonemura et al., 2010). Change in actomyosin contractility or tension at the AJ alter the conformation of α-catenin allowing it to interact with vinculin and thus strengthening the link between the AJ and the actin cytoskeleton (Yonemura et al., 2010).

Phylogenetic studies indicate αN-catenin is the common ancestor of αE- and αT-catenins (Hulpiau et al., 2013; Zhao et al., 2011). αE-catenin arose from a vertebrate-specific subphylum duplication whereas αT-catenin resulted from an amniote-specific gene duplication event. The functional significance of this latest *CTNNA* gene duplication will be discussed later in light of a specialized junctional complex recently identified in hearts of higher vertebrates. Interestingly, α-catenin predates cadherin as it was recently identified in the non-metazoan *Dictyostelium discoideum* that lacks a cadherin homolog (Dickinson et al., 2011). Like metazoan α-catenin, *Ddα*-catenin bind and bundle actin filaments and bind the α-catenin-related protein Aardvark. Most importantly, knockdown of *Ddα*-catenin disrupted the polarized organization of the tip epithelium demonstrating the requirement for the catenin complex for epithelial polarity in *D. discoideum*, a function conserved in metazoans. The role of α-catenin in embryonic morphogenesis has been studied in invertebrates including *C. elegans* and *Drosophila* (Maiden and Hardin, 2011). This review highlights genetic studies in mice investigating the requirement of the different α-catenin subtypes in various tissues and the significance of α-catenins in human disease.

αN-catenin and CNS development

αN-catenin expression is restricted to the central nervous system (CNS) in mice, suggesting a unique role in mammalian brain development where cadherin function is required for normal synaptic activity (Takeichi and Abe, 2005). It was discovered that the spontaneous *cerebellar deficient folia* (*cdf*) mutation identified in a mouse colony at The Jackson Laboratory (Cook et al., 1997) is caused by a 150 kb deletion that includes the 3 end of the *Ctnna2* gene encoding the F-actin-binding site (Park et al., 2002). The *cdf* mutant mice exhibit cerebellar ataxia and other abnormal behaviors including a deficit in fear-potentiated startle. Another group reported that in a conventional knockout of *Ctnna2*, the majority of the mutant mice die within 24 hours after birth (Togashi et al., 2002). The phenotypes reported include abnormal migration of Purkinje cell precursors in the cerebellum (Park et al., 2002; Togashi et al., 2002). They also include impaired dendritic spine morphogenesis in the hippocampal neurons that causes the formation of unstable synaptic junctions (Abe et al., 2004; Togashi et al., 2002). Transgenic expression of αN-catenin was able to restore normal cerebellar architecture in the *cdf/cdf* mice thus confirming that deletion of *Ctnna2* was responsible for the *cdf* mutant phenotype (Park et al., 2002). Despite the widespread expression of αN-catenin in the brain, neuronal defects are restricted to specific regions in the αN-catenin mutant brain. αE- and/or αT-catenin may compensate, at least partially, for loss of αN-catenin in the brain. αE-catenin is primarily expressed in neural progenitors whereas αN-catenin is expressed later in differentiated neurons (Lien et al., 2006; Stocker

and Chenn, 2006). Like αN-catenin, αT-catenin is also expressed in the mouse cerebellum (Vanpoucke et al., 2004). To understand the overall requirement for α-catenins in the adult brain it will be necessary to generate neuronal-specific double and triple α-catenin knockout mouse models.

αE-catenin in cancer development

Originally identified as an αE-cadherin-associated protein in epithelial cells (Nagafuchi and Takeichi, 1989; Ozawa et al., 1989), it is now appreciated that αE-catenin is expressed in most if not all cell types including neuron and muscle. Germline deletion of *Ctnna1* in mice disrupts development of the trophoblast epithelium resulting in mutant blastocysts incapable of hatching from the zona pellucida and implanting in the uterus (Torres et al., 1997). Despite the presence of αE-cadherin/α-catenin complex at the plasma membrane, the mutant embryos are unable to generate a blastocoelic cavity. αE-cadherin-null embryos exhibit a similar trophectoderm defect (Larue et al., 1994). Taken together, these data support an essential role for αE-catenin in αE-cadherin-mediated adhesion in the early preimplantation embryo.

To bypass the requirement for αE-catenin in the early embryo, several groups have used Cre/lox technology to investigate its function in a tissue-specific manner during embryonic morphogenesis and in the adult (Table 1). The Fuchs group initially reported deleting *Ctnna1* in the mouse epidermis beginning at embryonic day 13.5 (E13.5) using the keratin14-Cre (K14-Cre) transgene (Vasioukhin et al., 2001). Newborn αE-cat$^{fl/fl}$; K14-Cre mice exhibit multiple defects including loss of large patches of epidermis and decrease in hair follicles. Despite the presence of αE-cadherin/α-catenin complexes at the plasma membrane, ultrastructural examination of the epidermis showed intercellular gaps with a decrease in desmosomes and tight junctions. Remarkably, dividing keratinocytes were not only observed in the basal but also the suprabasal layers leading to a thick, disorganized αE-cat-null epidermis. The partial loss of cell polarity, hyperproliferation, large multinucleated keratinocytes, and mitoses in multiple cell layers resembled squamous cell carcinoma in situ, a precancerous condition observed in humans. The proliferation phenotype is not simply due to a cell adhesion defect or injury response as desmoplakin knockout skin displayed similar epidermal separation and peeling phenotype but no increase in keratinocyte proliferation. Furthermore, it was shown that loss of αE-catenin caused sustained activation of the Ras-MAPK pathway, and Erk1/2 pathway inhibitors were capable of blocking the hyperproliferation of the epidermal keratinocytes *in vitro* (Vasioukhin et al., 2001). Depending on the cellular context, α-catenins can modulate different signal transduction pathways involved in cell growth and survival. In the developing CNS, deletion of *Ctnna1* at E10.5 using Nestin-Cre resulted in mice with enlarged brains that die between 2 and 3 weeks of age (Table 1)(Lien et al., 2006). Expansion of the cerebral cortex in the mutant embryos is due to increased proliferation and decreased apoptosis in neural progenitors. Similar to the αE-cat-null epidermis (Vasioukhin et al., 2001), the loss of cell polarity did not affect neuronal differentiation in the αE-cat-null brains. Using microarray gene expression analysis, the Vasioukhin group found that Gli1, a downstream effector of the Hedgehog (Hh) pathway, was upregulated in the αE-cat-null brains. They showed that administration of cyclopamine, an inhibitor of the Hh pathway, to

αE-cat$^{fl/fl}$; Nestin-Cre embryos at E12.5 rescue the hyperplasia and apoptosis abnormalities in the cerebral cortex. Together, these data suggest that αE-catenin can regulate proliferation of epidermal and neural progenitor cells via distinct signaling pathways.

The Hippo pathway is critical for controlling organ growth in *Drosophila* and vertebrates (Barry and Camargo, 2013; Halder and Johnson, 2011). The core Hippo pathway consists of a cascade that signals from kinase Mst1/2 (Hippo in flies) to kinase Lats1/2 (Warts in flies) to limit the activity of the Yes-associated protein (Yap, Yorki in flies), a transcriptional coactivator that binds to the TEAD transcriptional factors to induce expression of cell cycle regulators and other target genes. The discovery that Yap activity in the epidermis does not depend on the canonical Hippo pathway kinases led the Camargo group to examine alternative regulatory mechanisms (Schlegelmilch et al., 2011). To identify novel Yap regulatory proteins, Yap immunoprecipitation was performed on high-density keratinocyte cultures followed by mass spectrometry analysis. This screen identified αE-catenin as the most common interaction partner with Yap. αE-catenin binds indirectly to Yap via the adaptor protein 14-3-3, which was also identified in the Yap complexes by mass spectroscopy, suggesting a tripartite complex composed of αE-catenin, 14-3-3, and Yap. The cellular localization of Yap is very much dependent on the cell's interactions with its neighbors (i.e., low versus high cell density). In high-density keratinocyte cultures, Yap is no longer localized to the nucleus but primarily cytoplasmic along with co-localization with αE-catenin at AJs. Knockdown in cultured keratinocytes or genetic depletion of αE-catenin in epidermis (i.e. αE-cat$^{fl/fl}$; K14-Cre) caused Yap to translocate to the nucleus resulting in hyperproliferation. Conversely, overexpression of αE-catenin in low-density keratinocyte cultures caused relocalization of Yap from the nucleus to the membrane. Interestingly, knockdown of other AJ components such as αE-cadherin or α-catenin did not affect the localization or activity of Yap suggesting that Yap hyperactivation is not due simply to loss of AJ-mediated cell adhesion. Phosphorylation of Yap at Ser127 causes cytoplasmic retention of Yap and thus inhibits its ability to induce transcription of target genes. In cells with reduced αE-catenin, Yap was found to interact with the phosphatase PP2A suggesting that αE-catenin together with 14-3-3 may regulate Yap activity by protecting the inactive, phosphorylated form of Yap from activation by PP2A. Further studies are necessary to clarify the role of αE-catenin in Yap regulation, including the involvement of other Yap interacting proteins such as angiomotins (Moleirinho et al., 2014).

Given the role of αE-catenin in regulating normal tissue growth, it is not surprising that it is involved in aberrant growth associated with cancer. Using GFAP-Cre, the Vasioukhin group deleted *Ctnna1* in the hair follicle stem cell niche at postnatal day 2 resulting in mostly bald mice (Table 1)(Silvis et al., 2011). Over time the αE-cat$^{fl/fl}$; GFAP-Cre mice developed extensive skin lesions with inflammation and tumors that resembled human squamous cell carcinoma of the keratoacanthoma type. The inactivation of p53, which often occurs in human keratoacanthoma, led to completely penetrant, early-onset, multifocal keratoacanthoma in αE-cat$^{fl/fl}$; p53$^{fl/fl}$; GFAP-Cre mice without the skin inflammation. A siRNA screen was performed to identify signaling pathways involved in the αE-catenin-dependent inhibition of cell growth. Like the Camargo group (Schlegelmilch et al., 2011), the Vasioukhin group identified Yap as being required for the hyperproliferation. As

predicted, Yap was localized to the nucleus in the hair follicle cysts and tumors in the αE-cat$^{fl/fl}$; GFAP-Cre mice. Consistent with the mouse data, decreasing αE-catenin is associated with increased nuclear Yap in human keratoacanthoma tumors.

Loss of αE-catenin has also been reported in other types of cancer (Ding et al., 2010; Fu et al., 2010; Liu et al., 2007; Piao et al., 2014; Raftopoulos et al., 1998). Interstitial loss of all or part of the long arm of chromosome 5 is a frequent clonal chromosomal abnormality in human myelodysplastic syndrome (MDS, a preleukemic disorder) and acute myeloid leukemia (AML). It was reported that *CTNNA1*, one of 12 genes contained within the 5q deletion, is expressed at lower levels in individuals with MDS or AML (Liu et al., 2007). Analysis of a myeloid leukemia line containing the 5q deletion showed that the *CTNNA1* promoter of the retained allele is suppressed by both methylation and histone modification. Restoration of αE-catenin resulted in reduced proliferation and apoptotic cell death. Together, these data suggest that loss of expression of the αE-catenin tumor suppressor in hematopoietic stem cells may provide a growth advantage that contributes to human MDS or AML with 5q deletion.

Emerging evidence suggests dual roles for αE-catenin in colon cancer. Mutation of the adenomatous polyposis coli (APC) tumor suppressor is an early step in most sporadic colon cancers, and APC mutations in inherited familial adenomatous polyposis (FAP) lead to early onset of the disease (Aoki and Taketo, 2007). Lost or reduced expression of αE-catenin is associated with colon cancer progression (Raftopoulos et al., 1998; Vermeulen et al., 1995; Vermeulen et al., 1999). Moreover, insertional mutagenesis in *Apc* mutant mice (i.e., Sleeping Beauty transposon system) identified *Ctnna1* as a common insertion site for promoting tumorigenesis in cooperation with APC (March et al., 2011). Interestingly, a different genetic study showed that αE-catenin is essential for the initiation of intestinal adenomas in *Apc*$^{580D/+}$ mice (Shibata et al., 2007). The *Apc* and *Ctnna1* genes are located in close proximity (~ 1 Mbp) on mouse chromosome 18. Deletion of one *Ctnna1* allele in the *Apc*$^{580D/+}$ background led to a decreased number of intestinal polyps compared to *Apc*$^{580D/+}$ with wild-type levels of αE-catenin. Researchers recently demonstrated that αE-catenin interacts with APC and facilitates α-catenin proteolysis through stabilizing the destruction complex thus repressing Wnt/α-catenin target gene expression (Choi et al., 2013). It remains to be determined how αE-catenin influences adenoma formation in the *Apc*$^{580D/+}$ mouse model. Additional mechanistic studies are required to understand the dual roles of αE-catenin in intestinal tumorigenesis, a supporting role in tumor initiation, and a suppressive role in tumor progression.

Downregulation of αE-catenin is also involved in the pathogenesis of basal-like breast cancer (Ding et al., 2010; Piao et al., 2014). Yap activation does not appear to be involved in this cancer type. Instead, αE-catenin was found to inhibit NF-kB signaling in αE-cadherin-negative basal-like breast cancer (Piao et al., 2014). Not normally thought of as tumor suppressor genes, mutations in *CTNNA2* and *CTNNA3* were recently identified in laryngeal squamous cell carcinoma (Fanjul-Fernandez et al., 2013) thus implicating all three *CTNNA* genes in tumor development.

α-catenins and mechanical coupling in the heart

The coordinated contraction of the heart depends on the proper mechanical and electrical coupling of cardiomyocytes. To achieve this goal cardiomyocytes are connected end-to-end by a specialized structure called the intercalated disc (ICD) that serves as an organizing center for various cell surface proteins including junctional complexes critical for cell-cell attachment and cell-cell communication. The ICD was reported to contain three distinct intercellular junctions: adherens junction (AJ), desmosome (Des), and gap junction (GJ) (Forbes and Sperelakis, 1985).

AJ and Des provide mechanical attachment between the myocytes by anchoring the actin cytoskeleton and intermediate filaments, respectively, at the ICD. GJs are plaques of multiple intercellular channels that connect the cytoplasm of adjacent cells. A major role of GJs in the myocardium is to enable the rapid and coordinated electrical excitation, a prerequisite for normal rhythmic cardiac function. It is well established from animal models (Peters et al., 1997) and human diseased myocardium (Peters et al., 1993) that altered gap junction expression referred to as gap junction remodeling contributes to arrhythmogenesis.

Until recently it was thought that AJ and Des represent distinct junctional complexes of the ICD. The desmosomal components expressed in the ICD include desmoplakin (DP), plakoglobin (PG), plakophilin2 (PKP2), desmocollin2 (DSC2), and desmoglein2 (DSG2). The idea of a mixed-type junctional complex as part of the normal heart structure was first suggested in 2006 (Franke et al., 2006). In this study, the authors revealed the presence of DP and PKP2 in desmosomes as well as "adherens junction-like" structures by immunoelectron microscopy. These 'hybrid adhering junctions' or 'areae compositae' contain both AJ and Des proteins and comprise the majority of intercellular junctions in heart muscle (Borrmann et al., 2006; Franke et al., 2006). Interestingly, the area composita is not found in lower vertebrates (Pieperhoff and Franke, 2008), which suggests that it might have evolved to support the increased mechanical load on the mammalian heart by anchoring both actin and intermediate filaments over an extended junctional area of the ICD. The area composita will be discussed later in the context of specific interactions between the AJ and Des components, αT-catenin and PKP2.

The importance of these adhesion molecules in the heart is highlighted by the fact that human mutations in genes encoding desmosomal proteins cause arrhythmogenic cardiomyopathy (AC), also known as arrhythmogenic right ventricular cardiomyopathy (ARVC), a hereditary heart muscle disease that causes sudden cardiac death (SCD) in young people and athletes (Thiene, 2012). The pathological features of AC consist of progressive loss of cardiomyocytes, myocardial degeneration, and compensatory replacement with fibro-fatty tissue. AC is considered a disease of the desmosome since about half of patients carry a mutation in one of the five genes encoding desmosomal proteins expressed in the heart (Rampazzo et al., 2014). A hallmark of AC is incomplete penetrance and variable expressivity of the disease phenotype making it difficult for clinicians to advise patients of their risk of SCD. Adding further to the genetic complexity AC patients were recently identified with more than one mutation in the same or different desmosomal gene,

suggesting that AC might require multiple genetic hits in the cell adhesion complex to elicit a cardiac phenotype (Bauce et al., 2010; Xu et al., 2010).

In the heart, there are two α-catenins expressed: the ubiquitously expressed αE-catenin and the largely cardiac-restricted αT-catenin. The cardiac-specific αE-catenin CKO model (αE-cat$^{fl/fl}$; MLC2v-Cre) presents with progressive left ventricular dilatation associated with a thinning right ventricular anterior wall leading to a high susceptibility to cardiac rupture following myocardial infarction (Table 1)(Sheikh et al., 2006). Loss of αE-catenin did not affect the expression of junctional components located in the area composita, Des, or GJ and no arrhythmias were reported in these mice. However, vinculin, a binding partner of αE-catenin, was decreased in the αE-catenin CKO heart. Another group reported significant mortality in αE-catenin heterozygous null mice following myocardial infarction (van den Borne et al., 2008) further supporting the importance of αE-catenin following ischemic injury.

Present only in higher vertebrates, αT-catenin is the newest member of the α-catenin family (Zhao et al., 2011). It is predominantly expressed in the heart and testis with lower expression in other tissues including the brain (Janssens et al., 2001). Analysis of the human *CTNNA3* promoter showed that cardiomyocyte expression is dependent on interaction of GATA4 transcription factor with a conserved 5 region of *CTNNA3* gene (Vanpoucke et al., 2004). Recent evidence suggests a unique role for αT-catenin in the formation of the hybrid junction or area composita in the heart. Using yeast two-hybrid and co-immunoprecipitation, αT-catenin was shown to bind the desmosomal protein PKP2 (Goossens et al., 2007a). By contrast, αE-catenin lacks PKP2 binding capacity. Importantly, immunoelectron microscopy demonstrated co-localization of αT-catenin and PKP2 in the area composita but not the Des. It is possible that the *CTNNA3* gene evolved, at least in part, to allow the formation of the hybrid adhering junction or area composita in the heart of amniotes (Pieperhoff and Franke, 2007; Pieperhoff and Franke, 2008). In addition, it is important to note that αT-catenin is found in amniotes that have a four-chambered heart while it is absent in amphibians that have a three-chambered heart. It is interesting to speculate that the septation of the ventricle in terrestrial vertebrates required a novel, more extended hybrid-type junction to support the mechanical load needed to effectively pump blood throughout the pulmonary and systemic circulations.

Characterization of an αT-catenin KO mouse model confirmed the link between αT-catenin and PKP2 in the area composita and its essential role in cardiac function (Li et al., 2012). In contrast to germline deletion of αE-catenin (Torres et al., 1997), αT-catenin-null mice are viable and fertile (Li et al., 2012). Loss of αT-catenin in the area composita leads to early onset of dilated cardiomyopathy, gap junction remodeling, and an increased susceptibility to ventricular arrhythmia in the setting of ischemia/reperfusion injury. The expression and distribution of area composita and Des components are not affected in the αT-catenin KO heart, with the exception of PKP2. The more severe cardiac phenotype in the αT-catenin KO compared to the αE-catenin CKO model reveals a unique role for αT-catenin in cardiac homeostasis (Li et al., 2012). The disruption of the αT-catenin-PKP2 interaction may affect the spatial organization of additional junctional components located in the area composita. The Delmar group has shown that PKP2 interacts with Cx43 as well as the sodium channel

Nav1.5 in cardiomyocytes (Oxford et al., 2007; Sato et al., 2011; Sato et al., 2009). Further characterization of the αT-catenin KO model is warranted to determine the molecular mechanism(s) responsible for arrhythmogenesis in these animals. The unique ability of αT-catenin to interact with PKP2 provides a new paradigm for understanding the molecular integration of the junctional components including GJs and ion channels.

Recently, two mutations in the human *CTNNA3* (*αT-catenin*) gene were identified in AC patients suggesting that perturbation of the area composita may play a critical role in the etiology of this disease (van Hengel et al., 2013). One *CTNNA3* mutation found in this screen of 76 AC patients inhibits the interaction between αT-catenin and β-catenin leading to a mislocalization of αT-catenin into the cytoplasm of HL-1 myocardial cells. The second *CTNNA3* mutation increases dimerization of αT-catenin, which might create aggresomes and disturb its function. This is the first time a cell adhesion molecule outside the desmosome has been implicated in the etiology of AC. Further studies in animal models are necessary to elucidate the consequences of the reported *CTNNA3* mutations in the working myocardium.

α-catenins and cardiac regeneration

Study of the growth patterns of rodent cardiac myocytes during early postnatal period demonstrates that myocyte number reaches a peak at 4 days of age, remaining unchanged thereafter (Li et al., 1996). At this time point, myocyte volume and binucleation increase leading to enlargement of the heart via hypertrophic growth. Binucleation results from DNA replication with karyokinesis but not cytokinesis. During the fetal and early postnatal period the cardiomyocyte elongates, myofibrils align, and maturation occurs resulting in a rod-shaped cardiomyocyte. During this morphological progression the αN-cadherin/catenin complex, initially distributed all along the cell borders, becomes restricted to the polarized ends of the cell to form the mature ICD (Fig. 2)(Hirschy et al., 2006). Interestingly, the redistribution of the N-cadherin/catenin complex to the ICD coincides with cell cycle withdrawal and differentiation of cardiomyocytes during the postnatal period (Li et al., 1996; Soonpaa et al., 1996), suggesting a role for areae compositae in myocardial growth control.

In support of this idea, it was recently reported that interfering with area composita proteins αE- and αT-catenin in the neonatal heart (αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; MHC-Cre) perturbs ICD maturation and causes sustained cardiomyocyte proliferation in the adult heart (Table 1)(Li et al., 2014). It was shown that α-catenins are required for the proper organization of the N-cadherin/catenin complex at the ICD in αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; MHC-Cre (α-cat DKO) cardiomyocytes. The hyperproliferation phenotype resulted in an increased number of cardiomyocytes in both postnatal day 7 and adult α-cat DKO hearts, a time period when cardiomyocyte cytokinesis has normally ceased. Loss of α-catenins led to translocation of Yap to the nucleus and increased expression of cell cycle genes. Like in epithelial cells, these data show that α-catenins can regulate Yap cellular distribution and activity in heart muscle. The cardiac phenotype depends on the developmental period when the *Ctnna1* and *Ctnna3* genes are deleted. Interestingly, deletion of both *Ctnna* genes in the adult heart when the ICD is already formed does not stimulate cardiomyocyte proliferation (αE-cat$^{fl/fl}$; αT-

cat$^{fl/fl}$; MHC-MerCreMer). This model, referred as IN-DKO, requires the administration of tamoxifen to the animal in order to induce deletion of *Ctnna1* and *Ctnna3* genes specifically in adult heart muscle. The presence of an established mature ICD structure in the adult heart may explain why ablation of both α-catenins at that time is not sufficient to elicit the proliferation phenotype. In comparison, deletion of both *Ctnna* genes during cardiac morphogenesis (αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; Tnnt2-Cre) causes embryonic lethality around mid-gestation (Radice, G. unpublished data). Further studies are necessary to characterize the embryonic lethal phenotype in this model.

It was reported that altered αN-cadherin expression and ICD remodeling occurs in the border zone of infarcted rat hearts (Matsushita et al., 1999). In another study, αE-catenin was reported to be preferentially downregulated in both the remote and infarct area of human hearts (van den Borne et al., 2008). Interestingly, inactivation of α-catenins in mice subjected to myocardial infarction induced cardiomyocyte regeneration and improved heart function (Li et al., 2014). The increase proliferation was accompanied by an increase in Yap-positive cardiomyocyte nuclei in the border zone and infarct zone in the α-cat IN-DKO. Whether Yap regulation by α-catenins is mechanistically similar between epithelial cells and heart muscle is not known. Future studies investigating details of these interactions would provide important insights into mechanisms underlying α-catenin/Yap-mediated cardiac regeneration.

αT-catenin function outside the heart

In addition to heart muscle, αT-catenin is expressed in testis, brain, and skeletal muscle (Janssens et al., 2001). Despite its high expression in testis, male mice carrying a mutation in *Ctnna3* are fertile (Li et al., 2012). A testis-specific alternative transcript (AT-X) was discovered that encodes for a truncated αT-catenin protein (70 kDa) referred to as isoform-X (Goossens et al., 2007b), which might explain the normal spermatogenesis observed in the αT-cat-null mice (Frans van Roy, personal communication). The original *Ctnna3* mutant allele contains a deletion of exon 3 resulting in loss of the full-length αT-catenin protein (Li et al., 2012). However, the putative AT-X promoter, located in intron 6, transcribes a novel exon X and the remainder of the murine *Ctnna3* gene resulting in isoform-X. Despite the absence of the full-length αT-catenin protein, the presence of isoform-X likely explains the normal spermatogenesis in the αT-cat-null mice (Frans van Roy, personal communication). Interestingly, the truncated isoform-X lacks the N-terminus α-catenin binding site and its expression is restricted to elongating spermatids. The functional significance of this expression pattern may relate to the fact that isoform-X binds l-afadin strongly whereas the full-length αT-catenin protein interacts weakly with l-afadin. Importantly, the l-afadin protein is involved in formation of another cell-cell adhesion complex, the nectin/afadin/ponsin (NAP) complex, present in the testis. Interestingly, loss of the nectin-2 part of the NAP complex perturbs interaction between Sertoli cells and elongated spermatids and results in defective sperm and infertility in mice (Mueller et al., 2003). It will be interesting to determine whether isoform-X regulates Sertoli-germ cell interactions via the NAP adhesion complex. To understand the functional relevance of truncated isoform-X in male germ cell maturation it will be necessary to generate isoform-X specific knockout mice.

The genetic mechanisms behind common complex diseases such as asthma are derived from multiple genes with minor effects. Genome-wide association (GWA) studies screening hundreds of thousands of single-nucleotide polymorphisms (SNPs) simultaneously using microarray systems have proved useful for identifying genetic changes that contribute to complex diseases. *CTNNA3* is one of the largest genes in the human genome with 18 exons spanning 2.3 Mbp on chromosome 10q21 (Janssens et al., 2003). Two independent GWA studies identified multiple polymorphisms of *CTNNA3* associated with increased susceptibility to toluene diisocyanate-induced asthma in Korean (Kim et al., 2009) and Canadian (Bernstein et al., 2013) workers. One of the *CTNNA3* polymorphisms associated with occupational asthma is also associated with childhood asthma and response to therapy (Perin and Potocnik, 2014). Based on αT-catenin expression pattern, it is unclear how this largely cardiac-restricted α-catenin isoform might affect lung physiology. Interestingly, it was recently discovered that αT-catenin is expressed in lung within the cardiac sheath of pulmonary veins (Folmsbee et al., 2014). The same group found that αT-cat-null mice have altered lung mechanics demonstrated by increased pressure-volume curve area suggesting loss of αT-catenin affects lung hysteresis. Moreover, the Tcat-null lungs show increased hyperresponsiveness to chemical challenge. These data suggest that αT-catenin may contribute to asthma through a mechanism independent of inflammation and related to cardiac and pulmonary vein dysfunction.

Other GWA studies have associated *CTNNA3* polymorphisms with late onset Alzheimer's disease (LOAD) (Ertekin-Taner et al., 2003; Lincoln et al., 2013; Martin et al., 2005; Miyashita et al., 2007; Myers et al., 2000). These GWA data are complicated because embedded in the intronic sequence of the large *CTNNA3* gene is a gene encoding leucine rich repeat transmembrane protein3 (LRRTM3). Importantly, LRRTM3 is involved in amyloid metabolism (Majercak et al., 2006). Like CTNNA3, LRRTM3 is a synaptic protein, therefore both *CTNNA3* and *LRRTM3* are positional candidate LOAD risk genes. αT-catenin is expressed in neurons where it localizes to the synapse as part of the cadherin/catenin complex, and thus it is interesting to speculate that altering αT-catenin expression and/or function might affect neuronal connectivity and survival in aging human brains. Further analysis of αT-catenin mouse models is warranted because it may provide phenotypic data to support *CTNNA3* as a risk gene for Alzheimers and pre-eclampsia (van Dijk et al., 2010).

Concluding remarks

The first indication that αE-catenin has other functions in the cell, besides anchoring the cadherin/catenin complex to the actin cytoskeletal network, came from conditional knockout studies in the mouse epidermis (Vasioukhin et al., 2001). The surprising hyperproliferation phenotype in the cat-null epidermis has now been observed in other cell types including neural progenitors (Lien et al., 2006) and cardiomyocytes (Li et al., 2014). In the absence of α-catenins, different signaling pathways likely converge to stimulate cell cycle activity with Yap as a major contributor to the proliferative phenotype. Although biochemical assays have identified α-catenin (Schlegelmilch et al., 2011; Silvis et al., 2011) and catenin (Radice, G. unpublished data) as novel binding partners with Yap, there is no consensus regarding how α-catenins control Yap cellular localization and activity. The mechanism is likely independent of the canonical Hippo signaling pathway. Interestingly, actin cytoskeleton

remodeling and tension can also regulate Yap nuclear translocation and activity although the molecular mechanism is poorly understood (Halder et al., 2012). As cytoskeletal modulators, α-catenins are good candidates to control Yap activity by modifying intercellular and intracellular tension mediated through AJs and the underlying cytoskeleton.

The three mammalian α-catenin subtypes exhibit overlapping yet distinct expression patterns that might complicate interpretation of knockout phenotypes particularly in the CNS where E-, T-, and αN-catenin are all expressed to some degree. In the heart, the characterization of single and double knockout α-catenin models has provided important insight into α-catenin subtype specific functions. αT-catenin is the only α-catenin that contains a PKP binding domain. This binding domain allows it to serve as a molecular integrator between AJs and Des at the area composita, a unique junctional complex found exclusively in the myocardium of higher vertebrates (Goossens et al., 2007a). As might be predicted, in comparison to αE-catenin, depletion of αT-catenin affected to a greater extent the structural integrity of the heart; this was demonstrated by earlier onset of cardiomyopathy and susceptibility to arrhythmias (Li et al., 2012; Sheikh et al., 2006). Notably, mutations in human *CTNNA3* have been identified in AC patients (van Hengel et al., 2013) consistent with an important role for αT-catenin in ICD organization and function.

In addition to the hyperproliferation phenotype in the αE- and αT-catenin DKO hearts, further studies are necessary to determine how loss of α-catenins affects intercellular adhesion and mechanotransduction in the heart, a tissue under significant mechanical load. Since α-catenins are known to function as mechanosensors, it will be of interest to determine the response of the α-cat DKO mice to different cardiac stress such as α-adrenergic stimulation. During cardiac regeneration, an essential step in the de-differentiation of adult cardiomyocytes is cardiomyocyte detachment from its neighbors and disassembly of their sarcomeric structure to facilitate cell cycle reactivation. In addition to regulating Yap activity, loss of α-catenins may contribute to regeneration by weakening the area composita thus facilitating disassembly of the ICD and myofibrils resulting in proliferation of adult cardiomyocytes in the infarct zone and border zone of the ischemic area. Functional interference with α-catenins or its downstream targets in the heart may represent a novel mechanism for enhancing signaling pathways beneficial in cardiac repair.

The *in vivo* consequences of depleting α-catenins depend very much on the state of maturation of the ICD in the cell at that particular time. This is best illustrated in the heart where both αE- and αT-catenin have been simultaneously depleted at different stages of heart development (Li et al., 2014). The α-catenin DKO phenotype is most severe when αE- and αT-catenin are both depleted during early cardiac morphogenesis resulting in embryonic lethality consistent with the importance of α-catenins in mediating cytoskeletal remodeling during morphogenesis. In contrast, depletion of α-catenins in the adult myocardium when the ICD is already formed has little if any consequence on tissue architecture. In comparison, simultaneous deletion of *α-catenin* (*Ctnnb1*)and *α-catenin* (*Jup*) in adult heart muscle results in loss of αN-cadherin, disassembly of the ICD, and SCD (Swope et al., 2012). Taken together, the αE-/αT-catenin and α-/α-catenin DKO models illustrate the different functional requirement of catenins in the αN-cadherin/catenin adhesion complex in the adult heart. Moreover, cardiac-specific depletion of vinculin, a major effector of α-

catenin mechanosensing, does not cause hyperproliferation (Zemljic-Harpf et al., 2007) indicating loss of the α-catenin/vinculin interaction at the AJ is not likely responsible for the increase in Yap activity.

Although α-catenin and α-catenin were discovered together as cadherin-associated proteins 25 years ago, α-catenin with its Wnt connection went on to become the darling of the cadherin/catenin complex. Well overdue, it is now α-catenin's time in the spotlight.

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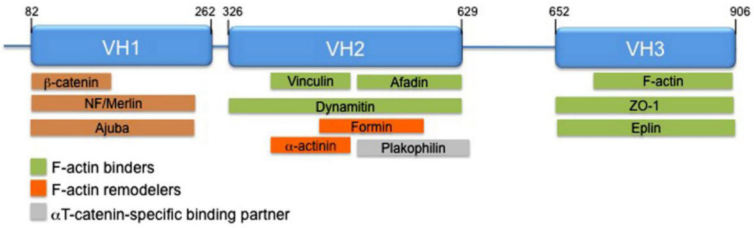

Figure 1. Schematic of α-catenin and its interacting partners

α-catenin contains three vinculin homology domains (VH1-3). α-catenin modulates actin assembly and dynamics directly and indirectly by acting as a scaffold for various actin regulatory proteins shown below the α-catenin structure. Note the plakophilin-binding domain is only present in αT-catenin.

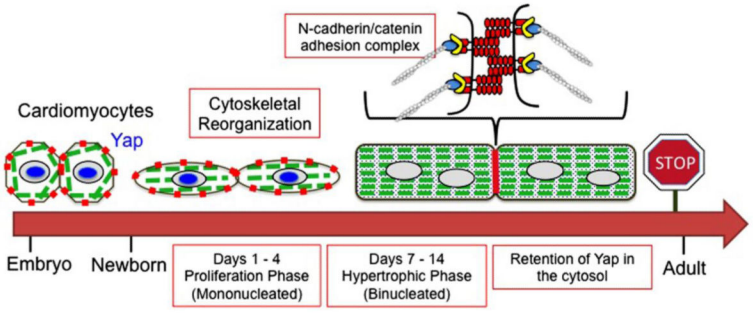

Figure 2. Schematic representation of heart muscle development in rodents

A timeline depicts changes in cardiomyocyte morphology, myofibril organization, intercalated disc maturation, and growth properties. Notably, intercalated disc formation in the postnatal heart coincides with loss of nuclear Yap and cell cycle withdrawal or STOP in proliferation. N-cadherin/catenin complex (red), myofibrils (green), Yap (dark blue), α-catenin (yellow), α-catenin (light blue).

Table 1

Genetic manipulation of α-catenin proteins in mice

Mutation	Tissue	Phenotype	Reference
αE-cat$^{-/-}$	global	Preimplantation embryonic lethal, trophectoderm cell adhesion defect	(Torres et al., 1997)
Cerebellar deficient folia (cdf/cdf) αN-cat deletion	global	Viable, ataxia, cerebellum and hippocampal lamination defects, abnormal startle response	(Park et al., 2002)
αN-cat$^{-/-}$	global	Perinatal lethality, aberrant cellular organization in the cerebellum and hippocampus, defective Purkinje cell migration, poorly formed dendritic spines	(Togashi et al., 2002; Uemura and Takeichi, 2006)
αT-cat$^{-/-}$	global	Viable, progressive cardiomyopathy, gap junction remodeling, susceptibility to arrhythmia	(Li et al., 2012)
αT-cat$^{-/-}$	global	Abnormal lung mechanics, increase sensitivity to toluene diisocyanate-induced asthma	(Folmsbee et al., 2014)
αE-cat$^{fl/fl}$; K14-Cre	skin	Cell-cell adhesion defect, loss of cell polarity, keratinocyte hyperproliferation	(Vasioukhin et al., 2001)
αE-cat$^{fl/fl}$; GFAP-Cre	hair follicle	Hair loss, skin squamous cell carcinoma	(Silvis et al., 2011)
αE-cat$^{fl/fl}$; nestin-Cre	neural tube	Hyperproliferation of neural progenitor cells; enlarged cerebral cortex	(Lien et al., 2006)
αE-cat$^{fl/fl}$; Emx1-Cre	cerebral cortex	Radial glial cell polarity defects	(Schmid et al., 2014)
αE-cat$^{fl/fl}$; Six3-Cre	retina	Defect in optic fissure closure, retinal degeneration	(Chen et al., 2012)
αE-cat$^{fl/fl}$; MLC2v-Cre	embryonic heart	Progressive cardiomyopathy, susceptibility to wall rupture after myocardial infarction	(Sheikh et al., 2006)
αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; αMHC-Cre (DKO)	perinatal heart	Intercalated disc defects, cardiomyocyte hyperproliferation	(Li et al., 2014)
αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; αMHC-MerCreMer (DKO)	adult heart	Enhanced cardiac regeneration following ischemic injury	(Li et al., 2014)
αE-cat$^{fl/fl}$; αT-cat$^{fl/fl}$; Tnnt2-Cre (DKO)	embryonic heart	Embryonic lethal, midgestation	Radice, G. unpublished data
αT-cat knock-in Rosa26 locus	global overexpression	Viable, fertile, aberrant trophoblast invasion	(Tyberghein et al., 2012)

fl, floxed (loxP-flanked) allele; DKO, double knockout.