Diabetic cardiomyopathy: role of the E3 ubiquitin ligase

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Bai T, Wang F, Mellen N, Zheng Y, Cai L. Diabetic cardiomyopathy: role of the E3 ubiquitin ligase. Am J Physiol Endocrinol Metab 310: E473–E483, 2016. First published January 5, 2016; doi:10.1152/ajpendo.00467.2015.—Diabetic cardiomyopathy (DCM) is the leading cause of mortality in diabetes. As the number of cases of diabetes continues to rise, it is urgent to develop new strategies to protect against DCM, which is characterized by cardiac hypertrophy, increased apoptosis, fibrosis, and altered insulin metabolism. The E3 ubiquitin ligases (E3s), one component of the ubiquitin-proteasome system, play vital roles in all of the features of DCM listed above. They also modulate the activity of several transcription factors involved in the pathogenesis of DCM. In addition, the E3s degrade both insulin receptor and insulin receptor substrates and also regulate insulin gene transcription, leading to insulin resistance and insulin deficiency. Therefore, the E3s may be a driving force for DCM. This review summarizes currently available studies to analyze the roles of the E3s in DCM, enriches our knowledge of how DCM develops, and provides a novel strategy to protect heart from diabetes.

diabetes; diabetic cardiomyopathy; E3 ubiquitin ligase; etiology; ventricular remodeling

DIABETES PREVALENCE CONTINUES TO RISE, reaching epidemic levels. It was estimated that in 2014, 9.3% of the US population (approximately 29.1 million people) had diabetes, and 27.8% of them have gone undiagnosed (10). Patients with diabetes are at two to four times increased risk of suffering heart disease. Indeed, the main cause of mortality in diabetes is cardiovascular disease, accounting for more than 50% of death (66). Although concomitant factors such as hypertension and atherosclerosis contribute to cardiovascular disease and the high ratio of mortality in diabetes, diabetes itself is an independent risk factor for heart damage. In diabetic animals, cardiac dysfunction occurs without hypertension and coronary heart disease. In diabetic patients, the risk of heart failure is increased even after controlling for hypertension and ischemic heart disease. Elevated circulating cytokines and hormones, altered adrenergic tone, increased free fatty acid, and hyperglycemia enhance malignant effects on the heart and cause cardiac functional changes and structural damage that together constitute a distinct disease entity termed as diabetic cardiomyopathy (DCM).

More than 40 years have passed since DCM was first described (82); however, despite extensive research, how DCM develops remains largely unknown. Recently, researchers have focused on gene transcription to elucidate mechanisms of disease progression. Several transcription factors (TFs), including the nuclear factor-κB (NF-κB), the forkhead box-containing protein O subfamily (FoxOs), nuclear factor (erythroid-derived 2)-like 2 (Nrf-2), and peroxisome proliferator-activated receptor-α (PPARα), were reported to play vital roles in the development of DCM (2, 5, 56, 91). This has led to the emergence of a related question: how are these TFs regulated in DCM?

The E3 ubiquitin ligases (E3s) not only degrade substrates in a ubiquitin proteasome-dependent manner but also regulate activity of gene transcription at the posttranscription level (24). By regulating the activity of the above-listed TFs, the E3s can be linked to DCM. The E3s also contribute to insulin resistance and insulin deficiency by degrading the insulin receptor (IR) and the insulin receptor substrate (IRS) and regulating insulin gene transcription. What’s more is that the E3s are involved in cardiac apoptosis, hypertrophy, and fibrosis, which are the main cardiac structural changes associated with diabetes. These studies suggest that the E3s act as a causative factor of DCM. Up until now, although many molecular mechanisms have been proposed for DCM, there remains less attention paid to the roles of the E3s in DCM (8). Therefore, this review focuses on a neglected aspect of the progression of DCM, focusing on the roles of the E3s.

Brief Overview of DCM: Key Pathophysiological Features and General Underlying Mechanisms

DCM was first described by Rubler et al. (82) in 1972 based on postmortem findings in four diabetic patients suffering from heart failure without other comorbidities. Later, these findings were confirmed by large epidemiological studies (23, 37). Because diabetes synergistically works with concomitant obesity, hypertension, and dyslipidemia, it is difficult to distinguish between a pure diabetic etiology and other cardiovascular risk factors; thus the concept of DCM as an independent disease entity has remained imprecise, and some scholars...
refute that DCM exists at all (49). However, animal studies increasingly show that diabetes alone could cause cardiac functional and structural changes (22). In vitro cellular studies using high glucose and palmitate to mimic diabetes also provide similar results. These studies strongly support the existence of DCM.

It is appreciated now that in diabetic heart, insulin signaling is blunted; cardiomyocytes have to increase fatty acid oxidation to maintain ATP production, which is concomitant with enhanced reactive oxygen species (ROS) generation, all of which have adverse effects on cell function. ROS may damage DNA, proteins, membrane lipids, and components of the electron transfer chain, thereby reducing efficiency of oxidative phosphorylation and further increasing ROS production. At the beginning, intracellular antioxidants counteract the effects of enhanced ROS. Over time, these antioxidants are depleted and exhausted shortly thereafter. The cardiomyocyte then undergoes an energy crisis, apoptosis, and altered gene transcription. An early indicator of diabetes-induced changes, extracellular matrix remodeling (also described as collagen accumulation), helps to explain why diastolic dysfunction is an early sign of DCM and precedes the development of systolic dysfunction (12). As diabetes progresses, these processes are exacerbated, leading to significant apoptosis, fibrosis, and hypertrophy. Once hypertrophy can no longer compensate for cell loss due to apoptosis and infiltration of fibrosis, systolic dysfunction emerges, which marks the transition to the late and irreversible stage of DCM. In the clinic, DCM typically first presents as heart failure with preserved ejection fraction, with features similar to restrictive cardiomyopathy. DCM then evolves into heart failure with reduced ejection fraction similar to dilated cardiomyopathy (19). The prognosis in patients with systolic dysfunction is poor and is exacerbated further by concomitant diabetes (15).

Therefore, as illustrated in Fig. 1, the main pathophysiological features of DCM include the shift in metabolic substrates at the early stage, followed by enhanced ROS generation, altered intracellular calcium transport and contractile apparatus, and increased cardiomyocyte apoptosis and then cardiac myocyte hypertrophy and fibrosis, leading to end-stage cardiac diastolic and systolic dysfunction.

Understanding how diabetes damages the heart in animals can be translated to develop strategies to guard against clinical heart failure with concomitant diabetes in humans. Hyperglycemia, oxidative stress, lipotoxicity, cell death, autophagy, microRNA, and epigenetic changes have been proposed as mechanisms of DCM (8, 9). Based on these hypotheses, specific interventions were designed to treat DCM. However, antioxidant reagents or ROS scavengers failed to protect diabetic heart in the clinic (97). A strategy of tight glucose control also failed to decrease heart failure in diabetic patients (16). So a new theory to clarify the mechanisms of DCM is required. A better understanding of these mechanisms will protect the heart from diabetes.

Overview: The E3 Ubiquitin Ligases

The E3 is one component of the ubiquitin-proteasome system, which is composed of three components: the ubiquitin-activating enzyme (E1), the ubiquitin-conjugating enzyme (E2), and the E3. The three components act differently. E1 forms a covalent bond with ubiquitin in an ATP-dependent manner, then E2 transfers ubiquitin from E1 to itself, and subsequently, the E3 recognizes specific substrates and transfers ubiquitin from E2 to substrate lysine. The fate of ubiquitinated substrate varies depending on the amount of ubiquitin added. When the substrate is polyubiquitinated (i.e., >4 ubiquitins attached), it is recognized by the 26S proteasome, which subsequently degrades the substrate to peptide and recycles the ubiquitin (77). By contrast, when the substrate is monoubiquitinated, it remains with altered biological activity or function due to allosteric regulation. For example, monoubiquitination of FoxO4 promotes its nuclear translocation, thereby enhancing transcriptional activity (96). Until now, only one E1 enzyme has been identified, but almost a dozen types of E2 enzymes and hundreds of E3s have been identified. Consider-

Fig. 1. Stages of diabetic cardiomyopathy. ETC, electron transfer chain; ROS, reactive oxygen species; RNS, reactive nitrogen species; ECM, extracellular matrix; MMP, matrix metalloproteinases; β-MHC, β-myosin heavy chain; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide.

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ing that the E3s have specificity for their substrates, the number of E3s accounts for their multiple and complex functions (98).

To date, the E3s are divided into three classes according to structural similarities: the really interesting new gene (RING) finger proteins (the most abundant class of E3 enzymes), the U-box proteins, and the HECT-domain (homologous to E6-associated protein COOH terminus) proteins (70). HECT domain proteins directly catalyze the degradation of substrates through their HECT domain, whereas RING finger proteins function as adaptor proteins facilitating the degradation of subunits. Although some RING finger proteins act independently, others function as a part of multisubunit complexes such as the cullin RING ubiquitin ligases (CRLs), which cull or sort substrates for degradation (73).

**The E3 Ubiquitin Ligases Regulate Insulin Signaling and Glucose and Lipid Metabolism**

Two important issues in diabetes are insulin deficiency and insulin resistance. Table 1 lists several E3s known to be involved in diabetes. The E3s contribute to both insulin deficiency and insulin resistance via multiple mechanisms (Fig. 2).

The Krüppel-like protein Gli-similar 3 (Glis3) is a transcription factor that plays a vital role in islet β-cell development. It was reported that Glis3 mutant mice developed neonatal diabetes, as evidenced by hyperglycemia and lower insulin levels. The pancreas in Glis3 mutant mice showed dramatic loss of β-cells and duct cysts (36). In addition, Glis3 is also essential for normal function of β-cells. For optimal activation of the insulin promoter in islet β-cells, Glis3 expression and its binding to conserved Glis3 enhancer elements located within the proximal insulin promoter are required. Mutations in enhancer elements and small-interfering RNA-directed knockdown of Glis3 were found to diminish insulin promoter activation (110). Itch, a WW-domain-containing HECT E3, can inhibit Glis3-mediated transcription of the insulin gene, thus causing insulin deficiency (111). In addition, it is known that mitochondrial damage is a fundamental contributor to pancreatic β-cell failure in diabetes (34). Parkin, a member of E3, is an important player in the maintenance of normal mitochondrial function via mitophagy. If Parkin is disturbed, the β-cell dysfunction ensues. This notion was supported by a recent study (29) in which cytosolic p53 disruption of mitophagy through an inhibitory interaction with Parkin induces mitochondrial dysfunction in β-cells. The occurrence of mitophagy is maintained in STZ-treated p53<sup>−/−</sup> mice that exhibit preserved glucose oxidation capacity and subsequent insulin secretion signaling, leading to better glucose tolerance. However, these protective effects are lost when Parkin is deleted (29). More E3s are involved in the development of insulin resistance. The IR and IRS are specific substrates for several E3s, including suppressors of cytokine signaling protein 1/3, muscle-specific mitsugumin 53 (MG53), and cullin 7 (83, 86, 107). These E3s mediate degradation of IR or IRS, thus directly promoting insulin resistance.

In contrast, one member of E3s, pellino3, protects against high-fat diet-induced insulin resistance through downregulation of interleukin-1β (IL-1β), a critical proinflammatory cytokine that promotes insulin resistance and diabetes (88). Compared with normal mice, pellino3-knockout mice displayed exacerbated insulin resistance and higher levels of IL-1β, and both effects were ablated by IL-1 receptor antagonist (108). As to the mechanism, two TFs that regulate IL-1β expression, NF-κB and hypoxia-inducible factor-1α (HIF-1α), were examined. Yang et al. (108) found that in pellino3-knockout mice the activity and protein level of HIF-1α were increased, whereas its mRNA level remained unaltered, indicating that the effect of pellino3 on HIF-1α was due to modulation of protein stability. Finally, these authors found that pellino3 deficiency could increase K63-linked ubiquitination and stabilization of HIF-1α and the expression of its response genes (108).

Besides their effects on insulin signaling, some E3s also regulate lipid, glucose, and whole energy metabolism, which are important variables in diabetes. For example, constitutive photomorphogenic protein 1 (COP1) promotes lipolysis in adipose tissue by degrading acetyl-coenzyme A carboxylase (ACC) and fatty acid synthase (FASN), which are rate-limiting enzymes in fatty acid synthesis (78, 109). The ubiquitin protein ligase E3 component n-recognin 5 regulates gluconeogenesis by mediating phosphoenolpyruvate carboxykinase degradation (33). Casitas b-lineage lymphoma plays a vital role in whole body energy metabolism possibly by modulating the activity of peroxisome proliferator-activated receptor-γ coactivator-1α and 5′-adenosine monophosphate-activated protein kinase (AMPK) (64).

In summary, the E3s not only interfere with insulin signaling but also regulate lipid, glucose, and whole body energy metabolism; thus they could be therapeutic targets in diabetes and its cardiovascular complications.

**The Roles of E3 Ubiquitin Ligases in Diabetes-Induced Cardiac Remodeling**

Under diabetic conditions, the heart had to undergo a serial of remodeling both in cardiomyocytes and extracellular matrix to copy with the altered metabolism. The main changes in cardiomyocytes are hypertrophy and apoptosis, and the primary alteration in extracellular matrix is fibrosis. To date, there is no unifying molecular pathway to account for cardiac re-
modeling, whereas the E3s are related to all the cardiac changes listed above (Table 2), suggesting the E3s act as causative factor that drives the heart into diabetic phenotype, which is discussed in the following sections.

The E3 ubiquitin ligases mediate cardiac fibrosis. Myocardial fibrosis and collagen deposition are the main structural changes observed in DCM. It is known that transforming growth factor-β (TGFβ) plays a causal role in cardiac fibrosis (48), and the Smad proteins are signaling transducers downstream from TGFβ receptors. Three families of Smad proteins have been identified: receptor-activated Smad2 and Smad3 (R-Smads), whose nuclear translocation can activate transcription of a select set of profibrosis genes, common partner Smad4, and inhibitory Smad6 and Smad7 (I-Smads). Smad7 is a key regulator of TGFβ signaling through negative feedback loops (32). It forms a stable complex with the TGFβ receptor, thus inhibiting phosphorylation and nuclear translocation of R-Smads (54). Smad7 also inhibits TGFβ signaling in the nucleus by interacting with transcription repressors such as histone deacetylases or disrupting formation of the R-Smads-DNA complex (113). Overexpression of Smad7 in primary cardiac myofibroblasts significantly reduces collagen synthesis, concomitant with increased expression of metalloproteinase-2 (101). In contrast, a decreased level of Smad7 contributes to cardiac fibrosis (26, 100). Three members of the E3s mediate degradation of Smad proteins. The Smad ubiquitination regulatory factor 1 (Smurf1) and factor 2 (Smurf2) bind to TGFβ receptors through Smad7 (17, 40) and degrade the TGFβ receptor, thus blocking profibrosis signaling. Arkadia, another E3 ubiquitin ligase, induces the ubiquitination and degradation of Smad7 and its repressors c-Ski and SnoN, thus enhancing TGFβ signaling (45, 67). In Otsuka Long-Evans Tokushima Fatty rats that typically showed hyperglycemia after 18 wk of age (41), cardiac expression of TGFβ receptor was increased at 15 wk of age, concomitant with enhanced collagen deposition (63). Thus, interventions targeting Smurf1 or Smurf2 are promising strategies to block cardiac fibrosis in the early stage of diabetes.

The E3 ubiquitin ligases regulate cardiac hypertrophy. Cardiac hypertrophy is an adaptive change in heart mass due to the enlargement of cell volume. To date, two distinct phenotypes of cardiac hypertrophy have been recognized (27). Cardiac hypertrophy in response to stimuli such as pressure overload and adrenergic stimulation activates the calcineurin/nuclear factor of activated T cells (NFAT)-dependent signaling pathway, which leads to “pathological hypertrophy,” concomitant with maladaptive changes such as fibrosis and chamber dilation (105). By contrast, hypertrophy stimulated by exercise, insulin, or insulin like growth factor I (IGF-I) signaling is dependent on activation of protein kinase B, also known as
Akt, which activates downstream targets, including glycogen synthase kinase-3β and mammalian target of rapamycin to regulate protein synthesis and cardiac gene transcription, thereby increasing the size of myocytes and heart mass. This type of cardiac hypertrophy is a physiologically adaptive process and occurs without chamber enlargement or heart failure. Thus, calcineurin and Akt are vital molecules in cardiac hypertrophy, and both of them are substrates for the E3s.

Atrogin-1, a member of E3s, inhibits cardiac hypertrophy via interactions both with calcineurin and Akt (Fig. 3) (52, 53). Calcineurin can dephosphorylate NFAT, which enables its nuclear translocation, thus promoting transcription of hypertrophy-related genes such as atrial natriuretic peptide and β-myosin heavy chain. By forming a multiprotein complex, atrogin-1 catalyzes ubiquitin-dependent degradation of calcineurin, thereby inhibiting cardiac hypertrophy (52). Atrogin-1 also prevents Akt-dependent cardiac hypertrophy by activating FoxOs, which are downstream of Akt and inhibit Akt via feedback mechanisms (53). Transgenic mice with atrogin-1-overexpressed in heart displayed resistance to cardiac hypertrophy in response to insulin-like growth factor treatment, whereas atrogin-1-knockout mice exhibited exacerbated cardiac hypertrophy in response to voluntary running (53).

Besides atrogin-1, other E3s also regulate cardiac hypertrophy via different substrates (Table 2). For example, the ubiquitin ligase muscle RING finger-1 (MuRF1) protein inhibits cardiac “pathological hypertrophy” through modulation of the calcineurin/NFAT signaling pathway and protein kinase C (60, 89). MuRF1 is also reported to attenuate IGF-I-dependent cardiac hypertrophy by inhibition of c-Jun (99). In addition, in isolated cardiomyocytes, MuRF1 colocalizes with troponin I and reduces the level of troponin I by increasing its degradation in a proteasome-dependent manner (42). Another in vitro study shows that overexpression of murine double-minute 2 (MDM2), a member of E3, can inhibit cardiac hypertrophy induced by α-agonists (93). These effects may be attributable to the fact that MDM2 interacts with and degrades the serine protein T-cap, which is a key protein related to cardiac hypertrophy (92). Troponin I and T-cap are essential components of the cardiac sarcomere (31), and their degradations by the E3s may contribute to cardiac wall thinness in some pathological conditions, such as dilated cardiomyopathy and cardiac remodeling after myocardial infarction.

In type 1 diabetic mice, cardiac expression level of MDM2 was decreased (72). Consistent with this, RINm5F cells subjected to high glucose showed decreased expression level of MDM2 in a time-dependent manner (3), whereas expression level of COOH terminus of heat shock cognate 70-interacting protein-1 (Hsp70) was increased (72). Consistent with this, atrogin-1-knockout mice exhibited exacerbated cardiac hypertrophy in response to voluntary running (53).

Table 2. The E3s related to cardiac fibrosis, hypertrophy, and apoptosis

<table>
<thead>
<tr>
<th>Name</th>
<th>Substrate</th>
<th>Functions</th>
<th>Ref. No.</th>
</tr>
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<tbody>
<tr>
<td>Arkadia</td>
<td>Smad7</td>
<td>↑ Myocardial fibrosis</td>
<td>26</td>
</tr>
<tr>
<td>Smurfl</td>
<td>TGFβ receptor</td>
<td>↑ Myocardial fibrosis</td>
<td>102</td>
</tr>
<tr>
<td>Smurfl</td>
<td>TGFβ receptor</td>
<td>↓ Myocardial fibrosis</td>
<td>13</td>
</tr>
<tr>
<td>MDM2</td>
<td>T cap</td>
<td>↓ Hyper trophy</td>
<td>92</td>
</tr>
<tr>
<td>Atrogin-1</td>
<td>Calcineurin</td>
<td>↓ Hyper trophy</td>
<td>52, 53</td>
</tr>
<tr>
<td>Akt</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MuRF1</td>
<td>PKC troponin 1</td>
<td>↓ Hyper trophy</td>
<td>42</td>
</tr>
<tr>
<td>MDM2/4</td>
<td>p53</td>
<td>↓ Apoptosis</td>
<td>93, 106</td>
</tr>
<tr>
<td>CHIP</td>
<td>p53</td>
<td>↓ Apoptosis</td>
<td>47, 68</td>
</tr>
<tr>
<td>cIAP1</td>
<td>Caspase 3/9</td>
<td>↓ Apoptosis</td>
<td>115</td>
</tr>
<tr>
<td>cIAP2</td>
<td>Caspase 3/7</td>
<td>↓ Apoptosis</td>
<td>30</td>
</tr>
<tr>
<td>XIAP</td>
<td>Caspase 3</td>
<td>↓ Apoptosis</td>
<td>87</td>
</tr>
</tbody>
</table>

TGFβ, transforming growth factor-β; MDM2, murine double minute-2; MuRF1, muscle RING finger-1; CHIP, COOH terminus of heat shock cognate 70-interacting protein; XIAP, X-linked inhibitor of apoptosis. ∨, due to specificity in substrates, a lot of the E3s regulate cardiac fibrosis, hypertrophy, and apoptosis, which are main cardiac structural changes in the diabetic cardiomyopathy. ↑, Promote or increase; ↓, inhibit or decrease.

Fig. 3. Atrogin-1 inhibits 2 distinct types of cardiac hypertrophy. When hearts suffer diabetes, ischemia, and hypertension (left), they undergo “pathological hypertrophy” concomitant with fibrosis, apoptosis, and cardiac dysfunction, where the calcineurin/nuclear factor of activated T cells (NFAT) pathway plays a major role. Calcineurin dephosphorylates NFAT and enables its nuclear translocation, thus promoting transcription of hypertrophy-related genes like ANP and β-MHC. Atrogin-1 catalyzes ubiquitin-dependent degradation of calcineurin, thereby inhibiting cardiac hypertrophy. When hearts meet stimulus-like exercise, pregnant, insulin, and growth hormone (right), the Akt pathway is activated and initials the physiological hypertrophy. Atrogin-1 activates forkhead box-containing protein O subfamilies (FoxOs) that are downstream of Akt to turn on cardiac atrogenes. In addition, activated FoxOs inhibit Akt via feedback. Both cardiac atrogenes and inactivated Akt result in an inhibition of cardiac hypertrophy. Illustrations are based on data from previously published works (27, 52, 53).
protein (CHIP) was increased (44). The expression level of MDM4, an MDM2 homolog, was also altered in peripheral blood cells from diabetic patients (61). These findings suggest that the expression levels of E3s are altered in diabetes and may contribute to cardiac hypertrophy in diabetes.

The E3 ubiquitin ligases regulate cardiac apoptosis. Myocardial apoptosis is thought to be a tightly controlled self-protective process in response to severe and long-lasting stress (46). It is increasingly clear that the E3s regulate cardiac apoptosis via interaction with p53 and several members of the caspase family (Table 2).

p53 is a well-known proapoptotic protein in the heart (57, 58), and a higher level of p53 is found in several types of heart diseases, including the DCM (6, 69, 85). Besides proapoptosis, p53 also damages the heart by increasing mitochondrial oxygen consumption and ROS generation (69). In heart, three E3s target p53 as their substrates: MDM2, its homologue MDM4, and CHIP. Through degradation of p53, the three E3s inhibit myocyte apoptosis, thus protecting the heart from stressors, including hyperglycemia and diabetes (47, 68, 93, 106). However, p53 also shows antiapoptotic activity through activating the Bcl-2 family (65). Mice deficient in p53 exhibit an increased risk of cardiac rupture after myocardial infarction (62, 114), which indicates a beneficial role of p53 in heart. Therefore, more evidence is needed before the concept that the E3s can protect the heart from diabetes via inhibition of p53-dependent apoptosis is established. Meanwhile, it must be remembered that p53 inhibition is accompanied by a higher risk of cancer.

In addition, the E3s regulate apoptosis by modulating the activities of caspases, which play essential roles in apoptosis. Inhibitors of apoptosis protein (IAPs) were initially identified in viruses by their ability to inhibit apoptosis in infected cells (14). The IAPs have a conserved RING finger motif at the COOH terminus that has an E3 ubiquitin ligase activity. To date, a growing number of IAPs have been identified, including three in heart: two cellular IAPs (cIAP1 and cIAP2) and X-linked IAP (XIAP). Studies have found that cIAP1 targets caspase-3 and caspase-9 (115), cIAP2 caspase-3 and caspase-7 (30), and XIAP caspase-3 as their substrates (87), which accounts for rapid degradation of active caspase-3 in a proteasome-dependent manner (11). Overexpression of XIAP protects β-cell function and decreases the amount of human islets required to reverse hyperglycemia in diabetic mice (18). XIAP also protects the heart from ischemic injury, as evidenced by smaller infarct size, reduced apoptosis, and decreased level of cleaved caspase-3 (43). However, most studies into the roles of IAPs in apoptosis have been carried out in vitro, and in vivo evidence is currently lacking. Thus, the role of IAPs in diabetes-induced myocardial apoptosis remains unresolved.

The E3 Ubiquitin Ligases Regulate Vital Transcription Factors in DCM

With the advent of new methods and animal models, we have gained deeper insight in the mechanism of DCM at the transcription level. To date, several TFs, including PPARα, Nrf-2, FoxOs, and NF-κB, have been identified as playing vital roles in DCM, and their activities are regulated by the E3s, which provide support for the hypothesis that E3s play a causal role in DCM.

PPARα was the first identified TF that related to the genesis of DCM (20). Along with its target genes, PPARα is increased in hearts from preclinical models of diabetes. Transgenic mice with cardiac overexpression of PPARα exhibited increased fatty acid oxidation and reduced glucose utilization. PPARα transgenic mouse hearts show significant ventricular hypertrophy, lipid deposition, and contractile dysfunction, features also found in diabetic hearts. This indicates that PPARα acts as a driving factor in cardiac transition toward a diabetic phenotype.

Two types of E3s, MG53 and MuRF-1, act as upstream regulators of PPARα (56, 81). MG53 positively upregulates the expression level of PPARα, thus triggering a cascade of events that lead to DCM. In the study, researchers employed a mouse model of cardiac-forced overexpression of MG53. At 20 wk of age, MG53-transgenic mice exhibited profound cardiac hypertrophy and fibrosis, myocardial lipid accumulation, and contractile dysfunction, all reminiscent of DCM. In addition, loss and gain-of-function studies were conducted in vitro, firmly establishing PPARα as downstream to MG53 (56). In contrast, MuRF1 inhibits activity of PPARα by monoubiquitination and facilitates its nuclear export, thus blocking PPARα-induced gene transcription (81). Interestingly, the inhibitory effect of MuRF1 on PPARα is proteasome independent.

Nrf-2 is a TF that regulates expression of a set of antioxidant proteins, such as NAD(P)H quinone oxidoreductase 1, glutathione S-transferase, and heme oxygenase-1, thus protecting the heart in oxidative stress. Under baseline conditions, Nrf-2 is combined with Kelch-like ECH-associated protein 1 (Keap1), which acts as a substrate adaptor of the cullin3-Keap1-E3 ubiquitin ligase complex. This E3 ubiquitin ligase complex adds polyubiquitin chain onto Nrf-2 and targets it for proteasome-dependent degradation, thus maintaining a low level of active Nrf-2 (112). Following oxidative stress, multiple cysteine residues in Keap1, e.g., Cys 151 and Cys 273, undergo redox-dependent modifications, Keap1 loses its ability to anchor Nrf-2, and Keap1-dependent Nrf-2 ubiquitination and degradation are inhibited (112), causing increased Nrf-2 stability and activation of antioxidant genes. Oxidative stress is an important causal factor in DCM genesis; therefore, Nrf-2, together with its downstream genes, is thought to be a protective defense against DCM (90). It has been reported extensively that the protection from diabetes-induced cardiac damage with several compounds such as sulforaphane and hydrogen sulfide is dependent on Nrf-2 (2, 75). In late-stage diabetes, however, Nrf-2 expression is decreased, possibly due to enhanced degradation, which can be improved by a proteasome inhibitor, for example, MG132 (103). With the exception of preventing Nrf-2 degradation, modulation activity of Keap1 is another way to enhance Nrf-2 level. It enables Nrf-2 release from the cullin3 ubiquitin ligase complex (25, 80); thus it is a promising target to protect the heart from diabetes.

FoxOs are emerging as important TFs in the development of DCM, and their levels are consistently high in diabetic heart. Specific FoxO1 knockout in heart prevents high-fat diet-induced cardiac dysfunction and suppresses β-myosin heavy chain expression (79). In vitro, cardiomyocytes deficient in FoxO1 utilize glucose as the primary energy substrate and exhibit decreased intracellular lipid accumulation, which stands in contrast to diabetic cardiomyocytes. FoxO1 is also...
involved in insulin resistance and hyperglycemia-induced cell apoptosis via downregulation of IRS and activation of caspase-3 (5, 14). In addition, activation of FoxO3a is relevant to cell injury induced by diabetes or hyperglycemia (76, 94, 95). The FoxOs are thought to be downstream of Akt, as their dependent transcription is inhibited by Akt phosphorylation. In cultured cardiomyocytes, forced expression of either FoxO1 or FoxO3 can trigger Akt phosphorylation and increase its kinase activity. The FoxO-activated Akt subsequently interacts with and phosphorylates FoxO, providing feedback inhibition (71). In the diabetic heart, Akt signaling is blunted and FoxOs are activated, thus facilitating cardiac remodeling toward DCM. The activities of the FoxOs are also regulated by the E3 ubiquitin ligases. Several E3s, for example, MDM2, COP-1, and CHIP, can bind directly to the FoxOs, promoting their ubiquitination and degradation (21, 39, 51). Besides their proteolytic function, some members of E3s also regulate activity of FoxOs via monoubiquitination (7). For example, monoubiquitination of FoxO4 facilitates its entry into the nucleus and transcriptional activity, whereas deubiquitination by the Dub-Usp7 reverses this process (24).

NF-κB is another TF that plays vital roles in the pathogenesis of DCM (59, 103), and its activity is also regulated by the E3s (38), which further emphasizes the causal relationship between the E3s and DCM (Fig. 4).

**Therapeutic Potentials of the E3 Ubiquitin Ligases in DCM**

Up until now, therapies based on the E3s targeting DCM have been unavailable. Because the roles of E3s in DCM are elucidated, this therapeutic approach may gain more attention. Central elements in the pathogenesis of DCM are blunted insulin signaling and abnormal activities of several TFs, which are all tightly controlled by the E3s. Therefore, E3-based therapies are promising strategies for rescuing heart in diabetes. Currently, there is no study using an inhibitor or activator of the E3s to treat DCM, but several reagents used to rescue the heart from diabetes indeed regulate activities of the E3s.

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**Fig. 4.** The E3 ubiquitin ligase (E3s) regulates activities of nuclear factor (erythroid-derived 2)-like 2 (Nrf-2) and NF-κB, which play vital roles in the development of DCM via modulating transcription of their targeted genes. By forming a multiprotein complex (composed of Cul1 or Cul3, Rbx1, Skp1, and F-box proteins), the E3s can selectively degrade IkBα and Nrf-2. Keap1, Kelch-like ECH-associated protein 1. Illustrations are based on data from previously published works (38, 103, 112).

**Fig. 5.** The E3s are upstream of DCM. The E3s regulate several vital transcription factors in DCM. Propelled by these TFs, the cardiomyocytes gradually undergo hypertrophy, fibrosis, and apoptosis, eventually resulting in cardiac systolic and diastolic dysfunction, thereby advancing the development of DCM.
AMPK is an important molecule involved in DCM, and its activity is regulated by the E3s (116). AMPK activators protect the diabetic heart through multiple mechanisms, including increasing insulin sensitivity and glucose utilization, enhancing autophagy, and preserving endothelial function (28). Given that pharmacological activators of AMPK such as resveratrol and metformin can increase mRNA expression and protein levels of atrogin-1 and MuRF1 in heart (4), their beneficial effects on DCM are also likely due to modulation activities of the E3s. Epidermal growth factor receptor (EGFR) signaling is involved in the development of DCM (1), and regulation of this signaling is a way to protect heart from diabetes (50, 55). A natural compound, curcumin, and its synthesized analog C6 protect against DCM (74, 84, 104) and can downregulate EGFR expression levels via enhancing CHIP levels, a chaperone-dependent ubiquitin ligase (35). These studies hint that DCM-protective reagents modulate the activity of E3s, highlighting the causal relationship between the DCM and E3s.

Conclusions

DCM is the main cause of death in diabetic patients. Currently, there is no effective treatment for this condition. As discussed above, E3s are related to all aspects of DCM, including insulin metabolism, cardiac hypertrophy and apoptosis, cardiac fibrosis, and several vital transcription factors (Fig. 5). This review sheds light on DCM progression and suggests that the E3s may be a novel therapeutic target to treat DCM.

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DISCLOSURES

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AUTHOR CONTRIBUTIONS

T.B. and F.W. prepared figures; T.B. drafted manuscript; T.B., N.M., and L.C. edited and revised manuscript; Y.Z. and L.C. approved final version of manuscript.

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