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REVIEW

Caveolins in cardioprotection – translatability and mechanisms

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Translation of preclinical treatments for ischaemia-reperfusion injury into clinical therapies has been limited by a number of factors. This review will focus on a single mode of cardiac protection related to a membrane scaffolding protein, caveolin, which regulates protective signalling as well as myocyte ultrastructure in the setting of ischaemic stress. Factors that have limited the clinical translation of protection will be considered specifically in terms of signalling and structural defects. The potential of caveolin to overcome barriers to protection with the ultimate hope of clinical translation will be discussed.

LINKED ARTICLES

This article is part of a themed section on Conditioning the Heart – Pathways to Translation. To view the other articles in this section visit http://dx.doi.org/10.1111/bph.2015.172.issue-8

Abbreviations

ANT, adenine nucleotide transporter; Cav, caveolin; CR, caloric restriction; CSD, caveolin-scaffolding domain; eNOS, endothelial NOS; GLUT4, glucose transporter 4; IPC, ischaemic preconditioning; KO, knockout; mPTP, mitochondrial permeability transition pore; ROS, reactive oxygen species

Tables of Links

TARGETS	
Enzymes[a]	**Transporters**[b]
Akt	ANT, adenine nucleotide transporter
eNOS	GLUT4, glucose transporter 4
ERK	**Catalytic receptor**[c]
F_0F_1 ATP synthase	Insulin receptor
Glycogen synthase kinase-3β	**Ion channels**[d]
PI3K	K_{ATP} channels, $K_{ir}6.2$
PKC	**GPCR**[e]
Src kinase	Adenosine A_1 receptor

LIGANDS
Resveratrol
TNFα

These Tables list key protein targets and ligands in this article which are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Pawson *et al.*, 2014) and are permanently archived in the Concise Guide to PHARMACOLOGY 2013/14 ([a,b,c,d,c]Alexander *et al.*, 2013a,b,c,d,e).

Introduction

Myocardial infarction is a major cause of death in the United States. One of the most beneficial experimental interventions to produce cardiac protection is termed ischaemic preconditioning (IPC) where sublethal ischaemia protects from subsequent lethal injury (Murry *et al.*, 1986). Defining molecular events regulating IPC has potential implications for therapeutic strategies for ischaemic heart disease and broader implications for cardiac hypertrophy, heart failure, diseases that secondarily lead to cardiac dysfunction (e.g. diabetes, hypertension) and other conditions where the balance between cell death and survival is critical. Despite a considerable amount of data describing preconditioning signalling, a precise pharmacological target and therefore a specific therapeutic agent remains elusive. This review will focus on limitations of protective interventions in the heart and a novel observation that caveolae, lipid-rich membrane microdomains enriched in caveolin-scaffolding proteins, may be a means to break down these barriers to the translation of experimental cardiac protection to clinical practice.

What is the state of understanding of mechanisms leading to IPC?

Following the seminal discovery of IPC by Murry, Jennings and Reimer in 1986 (Murry *et al.*, 1986), two parallel ideas have developed over the last ~25 years to account for IPC and provide a path for therapeutic development. One is the *signalling* hypothesis which proposes molecules converge to change the biochemistry and metabolism of the cell to affect protection and the other is the *structural* hypothesis in which IPC provides a physical, structural resiliency to the cardiac myocyte. The ratio of published papers is skewed 100-fold in favour of investigations focused on the signalling hypothesis.

Many studies have described IPC as a promiscuous stimulus that involves the initiation of many shared and interconnected signalling pathways that ultimately converge upon the mitochondria to cause cell protection and survival (Hausenloy *et al.*, 2005; Hausenloy and Yellon, 2006). The signal transduction pathways involve sequential triggers, mediators and end effects that culminate in modulation of mitochondrial function (Juhaszova *et al.*, 2004; Hausenloy and Yellon, 2006). Three main mitochondrial phenomenon – (i) opening of mitochondrial ATP-sensitive potassium (K_{ATP}) channels; (ii) generation of a small burst of reactive oxygen species (ROS) and (iii) maintenance of the mitochondrial permeability transition pore (mPTP) – have all been linked to the cardiac protective effects of IPC (Auchampach *et al.*, 1992; Gross and Auchampach, 1992; VandenHoek *et al.*, 1998; 2000; Becker *et al.*, 1999; Yao *et al.*, 1999; Pain *et al.*, 2000).

Following their initial discovery of IPC, Murry *et al.* showed in 1990 that IPC delays ultrastructural myocardial damage (i.e. to membrane and mitochondria) during subsequent lethal ischaemia (Murry *et al.*, 1990). This led to exploration of the 'structural protective' hypothesis where IPC was shown to preserve membrane integrity and mitochondrial structure (Moolman *et al.*, 1995; Armstrong *et al.*, 2001). Although limited in number, some proposed effectors of this structural preservation have been postulated: the mPTP (Fang *et al.*, 2008), dystrophin and spectrin (Armstrong *et al.*, 2001; Kido *et al.*, 2004), ROS (Miyamae *et al.*, 2002) and K_{ATP} channels (Geshi *et al.*, 1998). This concept was expanded to include preservation of mitochondrial function and structure by IPC and postconditioning in heart and liver (Zhong *et al.*, 2000; Giovanardi *et al.*, 2009; Penna *et al.*, 2009; Quarrie *et al.*, 2012). What remained unknown was whether IPC prevented injury by directly modulating structure or if signalling events initiated by IPC activated membrane repair processes that helped to maintain membrane integrity during periods of stress. The latter possibility would suggest that the molecular signalling and structural protection afforded by IPC could be linked through some common factor. Preservation of cellular ultrastructure is a tightly regulated process that has at its core molecular signalling leading to membrane repair and dynamics in the sarcolemma and mitochondria (Donaldson *et al.*, 2009; Hausenloy and Yellon, 2010; Cao *et al.*, 2011; Gottlieb and Gustafsson, 2011; Mousley *et al.*, 2012). Finding a molecule that bridges signalling to preservation of cellular ultrastructure may provide a novel therapeutic target to protect against ischaemia-reperfusion injury.

Caveolae and caveolins: a bridge that provides a unifying feature to cardiac protection

Caveolae, or 'little caves', are cholesterol and sphingolipid-enriched invaginations of the plasma membrane (Palade, 1953) and are considered to be a subset of lipid rafts (Pike, 2003). Caveolins, the structural proteins essential for caveolae formation, are present in three isoforms (Chun *et al.*, 1994; Parton *et al.*, 1997). Caveolins have a 20 amino acid scaffolding domain (caveolin-scaffolding domain, CSD) that anchors and regulates proteins (Sargiacomo *et al.*, 1995; Feron and Balligand, 2006). Canonically, caveolin (Cav)-1 and Cav-2 were thought to be expressed in many cell types, while Cav-3 was found primarily in striated (skeletal and cardiac) muscle and certain smooth muscle cells (Song *et al.*, 1996). Such concepts are currently being challenged with the identification and description of the structural importance for Cav-3 in non-muscle cells (Niesman *et al.*, 2013) and the identification of a functional consequence of caveolin localized to a variety of cellular compartments (Head and Insel, 2007; Fridolfsson *et al.*, 2014).

The expression of caveolin isoforms in the heart has been hotly debated. It is accepted that cardiac myocytes express Cav-3, the muscle-specific isoform (Song *et al.*, 1996; Tang *et al.*, 1996), and that other cell types in the heart express Cav-1 and Cav-2. Recent studies have provided evidence for the existence of and a signalling role for Cav-1 in cardiac myocytes with respect to ischaemia-reperfusion injury and maintenance of cardiac gap junctions (Patel *et al.*, 2007; Yang *et al.*, 2014). It was previously thought that Cav-1 and Cav-2 form hetero-oligomers and Cav-3 forms homo-oligomers (Tang *et al.*, 1996; Scherer *et al.*, 1997) but more recent data indicate co-expression and interaction of Cav-2 and Cav-3 in neonatal cardiac myocytes (Rybin *et al.*, 2003) and interaction of Cav-1, Cav-2 and Cav-3 in adult cardiac myocytes

(Hagiwara *et al.*, 2002; Head *et al.*, 2006). Other findings show that cell type-specific environments may regulate the interaction of caveolins. Thus, in fibroblasts, Cav-1 and Cav-2, but not Cav-3, interact, whereas in myoblasts, all three caveolin isoforms co-immunoprecipitate (Capozza *et al.*, 2005) and in microglia Cav-1 and Cav-3 have very distinct roles depending on the metabolic and structural state of the cell (Niesman *et al.*, 2013). Studies of mice with knockout (KO) or transgenic overexpression of caveolins demonstrate that the expression of caveolin is both necessary and sufficient for the formation of caveolae. Recent studies have identified another protein, cavin, as a key component of caveolae although studies on the physiological and pathophysiological role of cavin in the heart are limited (Vinten *et al.*, 2005; Hill *et al.*, 2008; Liu and Pilch, 2008a).

Regulation of caveolae and caveolins is complex and may help explain the general role of this structural protein in regulation of a wide range of physiological cellular functions. Protein–protein interactions as a function of charge, size and/or steric factors may contribute to the localization of proteins within the caveolae (Yamabhai and Anderson, 2002; Nichols, 2003; Pike, 2005). In addition, being lipid-rich microdomains distinct from surrounding membranes, caveolae may facilitate lipid–protein interaction (Rothberg *et al.*, 1992; Park *et al.*, 2004). Lipid modification of proteins, in particular palmitoylation and myristoylation, contribute, for example, to the localization of G-protein signalling components in raft/caveolae domains (Ratajczak *et al.*, 2003; Razzaq *et al.*, 2004; Rodgers *et al.*, 2005; Kim *et al.*, 2006). As noted earlier, the CSD, a hydrophobic region in the cytoplasmic amino terminal tail that interacts with protein 'partners' through hydrophobic interactions, has been proposed as a critical region by which signalling proteins interact with caveolins (Chini and Parenti, 2004; Becher and McIlhinney, 2005), although this notion has been recently challenged (Collins *et al.*, 2012). Finally, caveolins can undergo post-translational modification that may be critical to regulating not only signalling proteins, but also the response to pathophysiology. Caveolins contain three C-terminal cysteine residues that contain putative palmitoylation sites (Cys^{133}, Cys^{144} and Cys^{156}) (Dietzen *et al.*, 1995). Cav-1 undergoes phosphorylation (at Tyr^{14}) (Rothberg *et al.*, 1992; Li *et al.*, 1996) and Src-mediated phosphorylation alters the properties of Cav-1, including its interaction with extracellular matrix proteins (Grande-Garcia *et al.*, 2007; Grande-Garcia and Del Pozo, 2008) and has other potential physiological functions (Patel *et al.*, 2007; Yang *et al.*, 2014). Although Cav-3 has a putative phosphorylation site, no studies have been published to identify this site or its functional significance. However, Cav-3 has been reported to be sumoylated which affects receptor desensitization (Fuhs and Insel, 2011). Thus, caveolae and caveolins appear to have a variety of ways to regulate cell function.

Cav-3 has been identified in the sarcolemmal membrane, transverse tubules (T-tubules), the I-band/A-band interface and localized with ryanodine receptors in myocytes (Ralston and Ploug, 1999; Scriven *et al.*, 2005). Caveolins are involved in many cellular processes including vesicular transport, cholesterol and calcium homeostasis (Fujimoto *et al.*, 1992; Fujimoto, 1993; Scriven *et al.*, 2002; Jones *et al.*, 2004; Peng *et al.*, 2004), signal transduction (Lisanti *et al.*, 1994; Steinberg and Brunton, 2001; Cohen *et al.*, 2004; Williams and Lisanti, 2004) and have been recently detected in the mitochondria (Li *et al.*, 2001; Fridolfsson *et al.*, 2012). Caveolins function as chaperones and scaffolds, recruiting signalling molecules to caveolae to provide direct temporal and spatial regulation of signal transduction (Shaul and Anderson, 1998; Williams and Lisanti, 2004). Caveolins can inhibit activity of signalling proteins by interaction of the CSD with a caveolin binding motif present in many proteins found in caveolae including endothelial NOS (eNOS) and ERK1/2 (Engelman *et al.*, 1998; Feron *et al.*, 1998; Kamoun *et al.*, 2006). Alternatively, caveolins can promote signalling via enhanced receptor-effector coupling or enhanced receptor affinity when caveolins are up-regulated or overexpressed (Feron and Balligand, 2006; Raikar *et al.*, 2006). This has led to the concept of a 'caveolar paradox' in which caveolins may produce direct allosteric inhibition of molecules such as eNOS under basal conditions but facilitate increased signalling upon agonist stimulation through compartmentation (Feron and Kelly, 2001; Feron and Balligand, 2006).

An emerging concept suggests that signalling molecules exist as multiprotein complexes, 'signalosomes', continuously forming and dissociating under basal or stimulated conditions (Feron and Balligand, 2006). Caveolins are thought to play an integral role in the dynamics of these multiprotein complexes. Specifically, in regard to signalling molecules involved in cardiac protection, many GPCRs including opioid (Head *et al.*, 2005) and adenosine receptors (Lasley *et al.*, 2000) localize to caveolae and co-immunoprecipitate with caveolins. Additionally, many of the signalling molecules involved in cardiac protection, including the Gα subunit of heterotrimeric G-proteins, Src kinases, PI3K, eNOS, PKC isoforms and ERK are known to bind with the scaffolding domain of caveolin and be regulated by caveolin (Krajewska and Maslowska, 2004; Ballard-Croft *et al.*, 2006). Caveolin is known to be a key component and activator of PI3K/Akt signalling (Fecchi *et al.*, 2006), a pro-cell survival pathway that plays a significant role in preconditioning in the heart.

Initial evidence implicating a role for caveolin in cardiac protection was confirmed by infusion of the CSD peptide of Cav-1 into ischaemic/reperfused hearts which resulted in recovery of cardiac function (Young *et al.*, 2001). It was later shown that ischaemia/reperfusion injury activates p42/44 and p38 MAPK, redistributes Cav-3 and down-regulates expression of Cav-1 (Ballard-Croft *et al.*, 2006). The critical links between caveolar structure, caveolin protein and cardiac protection have emerged from a series of studies conducted by our group showing that physical disruption of caveolae negated protection in adult cardiac myocytes (Patel *et al.*, 2006), loss of protection in Cav-1 and Cav-3 KO mice (Patel *et al.*, 2007; Horikawa *et al.*, 2008; Tsutsumi *et al.*, 2010a,b) and restoration of the preconditioning phenotype by cardiac-specific overexpression of Cav-3 (Tsutsumi *et al.*, 2008).

Why is clinical translation so difficult and is caveolin a potential solution?

Since the original discovery of IPC nearly three decades ago, numerous molecular mechanisms and a host of therapeutic

targets have been identified but not clinically translated. The root cause of this problem in clinical translation is likely to be complex. Simply put, the problem derives from the many complicating factors leading to a disconnection between the robustness of preclinical models, which are almost always performed in healthy young animals with no ongoing pharmacological treatments, or the various modifiers of protection, including age, sex, existing disease and drug treatment, that are present in patients. Could these complicating factors be somehow limited? The remainder of this review will explore the role of caveolin as a key feature to rescue protective pathways in pathophysiological settings.

Ageing

Why does the aged heart have decreased ischaemic tolerance?

As the population ages, there is an increasing challenge to preserve organ function in the face of disease and the well-known age-related changes in organ function and functional reserve. Age is the most important predictor of mortality in patients with ischaemic heart disease (Boersma *et al.*, 2000). Consistent with this clinical result, aged human atrial myocytes are not protected from ischaemic insults (Mio *et al.*, 2008). Studies with preclinical models also reveal an increased sensitivity and decreased tolerance to ischaemia-reperfusion injury in the aged heart (Headrick *et al.*, 2003; Willems *et al.*, 2005). Mechanisms that underlie this age-related deficit are not clear but are postulated to involve abnormalities in cellular signalling and mitochondrial function (Tani *et al.*, 2001; Lesnefsky *et al.*, 2006; Peart *et al.*, 2007) although other mechanisms, such as dysfunctional calcium homeostasis (Swynghedauw *et al.*, 1995), have been suggested.

Ageing also results in remodelling of mitochondria in terms of lipid content and membrane integrity (Pepe, 2005), defects in respiratory chain components (Lesnefsky *et al.*, 2001) and increased oxidant stress (Hagen, 2003) which ultimately lead to reduced capacity to exclude calcium, generate ATP and limit injury mediated by ROS (Jahangir *et al.*, 2001; Lakatta and Sollott, 2002; Lesnefsky and Hoppel, 2008). All of these factors may affect mPTP function. The molecular composition of the mPTP is controversial. Potential components of the mPTP have included the adenine nucleotide transporter (ANT) on the inner mitochondrial membrane and the voltage-dependent anion channel on the outer membrane, although genetic inactivation studies have suggested that neither of these components is necessary for mitochondrial permeability transition to occur (Bernardi, 2013). Cyclophilin D in the mitochondrial matrix also is thought to play a role in response to stress and the formation of the mPTP (Javadov and Karmazyn, 2007). A benzodiazepine receptor, hexokinase, and creatine kinase have also been proposed as regulators of the pore. Recent work has suggested the F_0F_1 ATP synthase forms a channel with properties similar to the functioning mPTP (Bernardi, 2013). It is unclear if cardiac protective agents act by inhibiting the opening of a preformed mPTP complex, a particular subunit of the complex, or the assembly or organization of the complex. Work in a model of protection has shown that increased phosphorylation of glycogen synthase kinase (GSK)-3β reduces the affinity of the ANT for cyclophilin D, suggesting that assembly of the complex is targeted by protective signals to limit mPTP opening (Nishihara *et al.*, 2007). Importantly, regulation of the pore is diminished with age (Jahangir *et al.*, 2001; Seo *et al.*, 2008), but the precise mechanism of modulation is unknown (Di Lisa and Bernardi, 2005). Age-related changes may involve, as with cellular signalling, altered organization and function of the mPTP leading to inefficiency and dysfunction. Data from *Drosophila* indicate that the only protein that shows age-associated increases in carbonyl modifications (an index of oxidative injury) is ANT, a change that results in loss of mPTP function, which is accelerated by pro-oxidant stimuli (Yan and Sohal, 1998).

The function of mitochondria is intimately connected to mitochondrial dynamics. Mitochondria are in equilibrium between fusion and fission events to maintain their morphology and function. When fusion is inhibited, mitochondria become fragmented resulting in reduced glucose oxidation, respiration and loss of mitochondrial membrane potential (Olichon *et al.*, 2003; Griparic *et al.*, 2004). When fission is inhibited, mitochondria become tubular and elongated (Stojanovski *et al.*, 2004). Fission is important for segregating irreversibly damaged mitochondria targeted for degradation. Excessive fission and lack of fusion result in loss of mitochondrial DNA, increased generation of ROS and loss of the mitochondrial network (Yaffe, 1999). Aged hearts have fewer mitochondria suggesting defects in fusion/fission.

Is caveolin a therapeutic target for reduced ischaemic tolerance with ageing?

The discussion thus far leads to two separate possibilities: (i) there is a potential ageing deficit that leads to an altered cardiac phenotype with age or (ii) the cellular environment created by ageing limits normal processes. Importantly, these are not mutually exclusive. Treatments aimed at restoring ischaemic tolerance in the aged myocardium must address the 'ageing deficit' and/or recreate a 'young environment' to alter not only cellular signalling but also restore dysfunctional mitochondria. From an experimental perspective, the only known external intervention to extend life in a number of species and reduce disease risk associated with ageing in primates and humans is caloric restriction (CR), an idea first conceptualized in 1935 (McCay *et al.*, 1935). CR is likely to activate or deactivate a number of pathways to extend lifespan and to enhance protective and repair processes. These pathways include mitochondrial function, dynamics and autophagy (Masoro, 2009). Interestingly, two reports suggest that CR prevents an age-related decline in Cav-1 expression in hepatic sinusoids (Jamieson *et al.*, 2007) and maternal CR elevates message for caveolin in the fetal cardiac left ventricle (Han *et al.*, 2004). Could these findings somehow indicate a role for caveolin in longevity?

Little is known regarding caveolin expression and ageing. Early studies of ageing in isolated senescent cells showed increases in caveolin expression (Volonte *et al.*, 2002; Cho and Park, 2005). However, in such studies, the concept of 'ageing' is contrived, as it is dependent on passage number. The concept that senescence is equivalent to ageing is flawed, as senescence is defined as an inability of cells to divide.

Cardiac myocytes contain high levels of caveolin and are senescent cells by definition (they do not undergo significant cell division) but not necessarily aged. Therefore, the role of caveolin in ageing must be considered from a cell-type and organ-specific perspective. Animal studies reveal organ-specific patterns of changes in caveolin expression with age. Importantly, a decrease in the expression of cardiac Cav-3 (Kawabe *et al.*, 2001) is observed as a function of age, a result we confirm in our preliminary data. Ageing results in dissociation of Cav-1 and Cav-3 from membrane caveolae (Ratajczak *et al.*, 2003). Cav-3 KO mice develop a progressive cardiomyopathy (Woodman *et al.*, 2002) and are also resistant to cardiac protective stimuli (Horikawa *et al.*, 2008). Cav-1-deficient mice show reduced lifespan and increased cardiac dysfunction (Park *et al.*, 2003) and are resistant to cardiac protective stimuli (Patel *et al.*, 2007). We have recently shown that ischaemic tolerance is reduced in human atrial tissue (Peart *et al.*, 2014). This observation was paralleled with the observation that Cav-3 is decreased in aged, compared with young, mouse hearts. We, furthermore, have indications that Cav-3 decreases with age in human hearts (unpublished data).

Caveolae are dynamic entities that form and dissipate in response to various stimuli (Tsutsumi *et al.*, 2008) and serve as a clathrin-independent mechanism for the endocytosis of plasma membrane constituents. Caveolae-mediated endocytosis facilitates transport of vesicles to other cellular regions and across the cell (transcytosis) (Mukherjee *et al.*, 2006; Ge *et al.*, 2008). Co-expression of flotillins 1 and 2 in caveolae enhances the accumulation of intracellular vesicles (Frick *et al.*, 2007). The fate of such vesicles is unknown. Recent data indicate that caveolae forms contacts with other cellular compartments to communicate membrane-derived signals to other organelles and regions of the cells. For example, smooth muscle cells have 'nanocontacts' between caveolae and the endoplasmic reticulum (Gherghiceanu and Popescu, 2007). Caveolins are found in cells and intracellular regions lacking caveolae, suggesting roles for caveolins in non-sarcolemmal locations (Head and Insel, 2007; Fridolfsson *et al.*, 2014). We have recently shown that there is a stress-adaptive transfer of caveolin to mitochondria which is facilitated by IPC that leads to protection of the heart from ischaemia-reperfusion injury and that this is a generalized protective pathway active in cancer and *Caenorhabditis elegans* and involves the activation of GPCR signalling and survival kinases (Fridolfsson *et al.*, 2012; Wang *et al.*, 2014). It is possible that a loss of caveolin expression with age affects the ability of the membrane not only to house and regulate survival kinases but also limits the ability of the cell to modulate mitochondrial function during stress.

Diabetes

Why is the diabetic heart dysfunctional?

According to the American Diabetes Association in the United States, there are nearly 26 million individuals, adults and children, with diabetes. In addition, there may be as many as 79 million individuals who are prediabetic. In 2007, diabetes was listed as the underlying cause of >70 000 deaths and a contributing factor of an additional 160 000 deaths. Those aged 65 years or older represent an ever-growing population facing the consequences of diabetes. In 2004, the most recent year for which statistics are available, heart disease was noted in nearly 70% of diabetes-related deaths among people 65 years or older and adults with diabetes have heart disease mortality rates that are two to four times higher than adults without diabetes.

Controversy exists as to whether cardiac events associated with diabetes are a consequence of underlying coronary artery disease and hypertension. Growing evidence suggests that diabetes results in altered cardiac structure and function independent of vascular pathology, supporting the existence of a 'diabetic cardiomyopathy'. Diabetes in animal models results in both diastolic (i.e. prolongation of relaxation and increased left ventricular end diastolic pressure) (Joffe *et al.*, 1999) and systolic (i.e. heart rate, systolic BP and fractional shortening) (Joffe *et al.*, 1999) dysfunction and such findings are also observed in humans (Poirier *et al.*, 2001). Structural changes also have been observed in the diabetic heart that include perivascular and interstitial fibrosis, possibly as a result of replacement of myocyte loss, altered mitochondrial structure and altered cardiac ultrastructure (Eto *et al.*, 1987; Warley *et al.*, 1995; Mizushige *et al.*, 2000). The molecular mechanisms proposed for diabetic cardiomyopathy are diverse and may include impaired calcium handling, altered substrate supply and utilization, altered energy generation with mitochondrial dysfunction, altered ion channel function, myocyte apoptosis, endothelial dysfunction, cardiac insulin resistance and activation of the renin-angiotensin system (Zhang and Chen, 2012). Additionally, diabetic hearts are refractory to protective interventions that limit ischaemia-reperfusion injury, suggesting major defects in survival kinase signalling (Balakumar and Sharma, 2012a). Such findings suggest that diabetic cardiomyopathy is a complex disease that is manifested with many cellular alterations that may or may not have a common control point of regulation that can be targeted therapeutically.

Are caveolins potential regulators of diabetes?

Cav-3 KO mice have a variety of deleterious phenotypes, such as muscle degeneration (Hagiwara *et al.*, 2000), insulin resistance (Oshikawa *et al.*, 2004) and progressive cardiomyopathy with age (Woodman *et al.*, 2002). Knockdown of Cav-1 in adipocytes results in loss of insulin receptor signalling as a result of decreased insulin receptor and glucose transporter 4 (GLUT4) expression (Gonzalez-Munoz *et al.*, 2009). Although hearts of Cav-1 and Cav-3 KO mice develop cardiomyopathy, they appear to have normal substrate utilization (Augustus *et al.*, 2008). In H9C2 cardiomyoblasts, Cav-1 knockdown has been shown to inhibit signalling by insulin-like growth factors (Salani *et al.*, 2008) and insulin signalling directly coupled to Akt and glucose transport (Ha and Pak, 2005). Importantly, Cav-3 was a positive regulator of insulin signalling (Yamamoto *et al.*, 1998) and caveolin gene transfer to the liver improved glucose metabolism in diabetic mice (Otsu *et al.*, 2009). Recently, our group has shown that Cav-3 overexpression in the heart leads to enhanced Akt phosphorylation that results in protection of the heart from ischaemia-reperfusion injury (Tsutsumi *et al.*, 2008). Other studies reveal that compounds such as resveratrol, which are

polyphenols shown to have lifespan-expanding properties (Frojdo *et al.*, 2008), also recruit GLUT4 to caveolae and up-regulate Akt signalling in the setting of type I diabetes (Penumathsa *et al.*, 2008). Caveolae also are major regulators of calcium storage and influx which may be an added cellular regulatory feature important to limiting diabetic cardiomyopathy (Shaul and Anderson, 1998).

Diabetes results in altered cardiac mitochondrial function with respect to complex activity, generation of ATP and activation of the mPTP, a key feature leading to cellular apoptosis (Oliveira *et al.*, 2003; Boudina *et al.*, 2007). Recent evidence suggests that caveolin-deficient stromal cells have compromised mitochondrial function (Pavlides *et al.*, 2010) and mitochondria from Cav-1-KO fibroblasts accumulate cholesterol and have severe dysfunction; such cells adapt poorly to nutrient starvation and are predisposed to apoptosis (Bosch *et al.*, 2011). Loss of caveolin leads to altered mitochondrial function in adipose tissue, suggesting a link between caveolin and metabolism (Wernstedt Asterholm *et al.*, 2012).

Caveolins also may play a role in pathologies associated with diabetes including metabolic syndrome, as recently reviewed by Zhang (2014). Specifically, the association between GLUT4 transporters and caveolin plays an important role in the development of insulin resistance (Kabayama *et al.*, 2007; Liu *et al.*, 2008b). Clinical studies utilizing caveolin as a marker or protein of interest in the setting of insulin resistance are rare and primarily address insulin resistance in the context of caveolinopathies (Mendez-Gimenez *et al.*, 2014). Some authors argue in favour of the importance of the caveolar structure, rather than the loss of either Cav-1 or Cav-3 (Mendez-Gimenez *et al.*, 2014). In a translational approach, Cav-1 polymorphisms have been linked to insulin resistance and hypertension in Caucasian and Hispanic patients (Pojoga *et al.*, 2011). In diabetes mellitus patients that underwent flow-mediated dilation of coronary arterioles during heart surgery, membrane localized Cav-1 was significantly reduced. This reduction was attributed to peroxynitrite, which contributes to microvascular dysfunction in diabetes mellitus (Cassuto *et al.*, 2014).

A major confounding factor in the translation of protective strategies to patients with diabetes is that many pharmacological agents that patients are prescribed may negate cardioprotection. Most diabetic patients receiving oral medications will be taking a sulfonylurea that blocks K_{ATP} channels, which in the pancreas increases insulin secretion but, in the heart, the same drug results in the attenuation of cardiac protection (Gross and Auchampach, 1992). Most diabetics and elderly patients are also on statins to maintain low blood cholesterol. Although statins have been shown to have many effects that have potential to protect the heart in specific settings, there is growing concerns that diabetics and individual with other pathophysiologies may not benefit as much as previously thought (Gullestad *et al.*, 2007; Drummond *et al.*, 2010; Schilling *et al.*, 2014). Reduction of caveolin through statin treatment in endothelial cells could affect protection indirectly, through modifying eNOS signalling (Balakumar *et al.*, 2012b). Conversely, one could argue that statin inhibition of the cholesterol pathway and a consequent decrease in Cav-3 in cardiac myocytes could result in impaired survival kinase signalling. The proof of this concept in the heart, specifically under long-term treatment, is still under investigation, while some effects of statin treatment on Cav-1 and the development of diabetes in preclinical models have been recently reviewed (Brault *et al.*, 2014).

In both the ageing and the diabetic heart, the two central features of the pathology are loss of effective signalling networks and compromised ultrastructure, the two perquisites Murry, Jennings and Reimer described early on, as being critical to the induction of IPC. Central to this dysfunction appears to be the loss of caveolin in the heart.

Caveolins in myopathies

Cav-3 interacts with signalling molecules involved in cardiac hypertrophy, remodelling and the progression of heart failure (Fujita *et al.*, 2001; Krajewska and Maslowska, 2004). Cav-3 KO mice exhibit reduced cardiac function and cardiomyopathy (Woodman *et al.*, 2002). These results suggest a potential role for Cav-3 in heart failure. Expression of cardiac Cav-3 is changed in models of heart failure and patients with cardiomyopathy, although there are inconsistencies in the findings (Hare *et al.*, 2000; Damy *et al.*, 2004; Hayashi *et al.*, 2004; Ruiz-Hurtado *et al.*, 2007). In the heart, mutations of Cav-3 can lead to familial hypertrophic cardiomyopathy (Hayashi *et al.*, 2004) as well as arrhythmias such as the congenital long-QT syndrome (Vatta *et al.*, 2006; Balijepalli and Kamp, 2008). In the models and patients examined, the variability of the results may be due to species differences or the stage of heart failure development and ventricular dysfunction. Recently, Feiner *et al.* (2011) reported reduced levels of Cav-3 in two well-established models of heart failure in mice, overexpression of the adenosine A_1 receptor or TNFα. They found significantly reduced levels of Cav-3 protein and mRNA in the mice with heart failure and showed a significant correlation between the reduced levels of Cav-3 and reduced cardiac function. In addition, these investigators found a significant correlation between the reduced levels of Cav-3 in failing human heart samples and the levels of the sarcoplasmic-endoplasmic reticulum calcium ATPase, a marker of heart failure. Our group has shown that cardiac myocyte-specific overexpression of Cav-3 limits the hypertrophic response to transverse aortic constriction and improves survival (Horikawa *et al.*, 2011). Such data indicate a role for Cav-3 as a therapeutic protein in heart failure. Cav-3 also plays a role in muscular dystrophies (Woodman *et al.*, 2004) including limb girdle muscular dystrophy type 1C (Angelini, 2004). The pathogenesis involved in these diseases involves a failure to traffic Cav-3 from the Golgi network to the plasma membrane (Woodman *et al.*, 2004).

Translational approaches to increasing caveolin

From the data presented, it is evident that heart-specific decreases in caveolin are detrimental to the heart, whereas up-regulation of caveolin may be beneficial to the heart. Our laboratory is currently developing a gene therapy-based approach to overexpress caveolin in a cell type-specific manner using selective promoters and regulatory elements.

Although this approach has clinical potential, translation is likely to be far in the future. Therefore, we need to find other natural means to increase caveolin expression. In one study on 14 male pentathlon athletes, exercise increased Cav-1, Cav-3, GLUT4 and the insulin receptor-β in samples from the vastus lateralis muscle after a 1500 m swim trial (Kim *et al.*, 2009). In another experiment, the manipulation of pre-exercise muscle glycogen storage was assessed. Here, an increase in the baseline levels of Cav-1 after recovery from initial glycogen depletion exercise was noted (Roepstorff *et al.*, 2004). Furthermore, in an exercise countermeasure during 12 weeks of bed rest, exercise altered NOS2/Cav-3 co-immunostaining patterns in vastus lateralis and soleus myofibres (Rudnick *et al.*, 2004). Additionally, in an intensive care unit model in rats, immobilization results in distinct alterations in gene expression and down-regulation of Cav-3 expression (Llano-Diez *et al.*, 2011).

Conclusion

Caveolae and caveolins are comparatively new players in a relatively saturated field of ischaemia-reperfusion injury. Given the data provided here, it should be clear that there is a central role for caveolin expression in the protection of the heart, and potentially other organs, from ischaemia-reperfusion injury and cell stress in general. It is intriguing that conditions in which protection is lost show marked loss of caveolin expression coupled to decreased survival kinase signalling and dysfunctional myocyte ultrastructure. Mice with cardiac specific overexpression have dramatic cardiac stress adaptation in a variety of disease settings and provide hope that caveolin may serve as a critical mediator and potential therapeutic target to provide protection from ischaemia-reperfusion injury in humans.

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Conflict of interest

There are no competing interests or disclosures.

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