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Review article

Reactive oxygen at the heart of metabolism

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ABSTRACT

During heart development, the progression from a pluripotent, undifferentiated embryonic stem cell to a functional cardiomyocyte in the adult mammalian heart is characterised by profound changes in gene expression, cell structure, proliferative capacity and metabolism. Whilst the precise causal relationships between these processes are not fully understood, it is clear that the availability and cellular ability to utilise oxygen are critical effectors of cardiomyocyte differentiation and function during development. In particular, cardiomyocytes switch from a largely glycolytic-based production of ATP to predominantly β-oxidation of long-chain fatty acids to generate the cellular energy requirements. Whilst this transition occurs progressively during embryonic and foetal development, it is particularly abrupt over the period of birth. In the adult heart, many cardiopathologies are accompanied by a reversal to a more foetal-like metabolic profile. Understanding the mechanistic causes and consequences of the normal metabolic changes that occur during heart development and those in the pathological heart setting is crucial to inform future potential therapeutic interventions. It is becoming clear that reactive oxygen species (ROS) play critical roles in the regulation of redox-mediated molecular mechanisms that control cellular homoeostasis and function. ROS are generated as a consequence of metabolic processes in aerobic organisms. An overproduction of ROS, when not balanced by the cell's antioxidant defence mechanisms (termed "oxidative stress"), results in non-specific oxidation of proteins, lipids and DNA and is cytotoxic. However, the tightly regulated temporal and spatial production of ROS such as H_2O_2 acts to control the activity of proteins through specific post-translational oxidative modifications and is crucial to cellular function. We describe here the metabolic changes that occur in the developing heart and how they can revert in cardiopathologies. They are discussed in the light of what is currently known about the regulation of these processes by changes in the cellular redox state and levels of ROS production.

Metabolism during early cardiogenesis

In mammals, the heart is the first organ to form to sustain the developing embryo with sufficient nutrients and oxygen (Buckingham et al., 2005). Therefore, commitment of embryonic stem cells (ES cells) to the cardiotypic lineage occurs early during embryogenesis (Kirby, 2002; Parameswaran and Tam, 1995). ES cells are noncontractile, requiring little ATP; thus, their transition to fully functional beating cardiomyocytes that require high levels of ATP to drive their sarcomeric pumps is metabolically dramatic (Chung et al., 2007). *In vivo*, cardiac progenitors derive from populations of mesodermal

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cells residing on either side of the midline in the lateral plate, which migrate and coalesce to form the cardiac crescent and subsequently the heart tube (Abu-Issa and Kirby, 2007). The myocardium then expands and loops to form the four-chambered heart. During this early stage of cardiogenesis, the cardiomyocytes are therefore proliferating rapidly (Christoffels et al., 2000). A proliferating cell will have high biosynthetic needs, and thus there will be a greater metabolic flux through anabolic rather than catabolic pathways (Lunt and Vander Heiden, 2011). Various cellular components such as nucleotides, proteins and lipids need to be synthesised in large amounts to increase biomass prior to cell division. Therefore, the need for macromolecular precursors is relatively higher than that for ATP. Thus the metabolic profile of proliferating cells is typically characterised by high rates of glycolysis and lactate production and relatively low rates of oxidative phosphorylation (Lunt and Vander Heiden, 2011).

In the mouse, the heart tube becomes a looped tube by embryonic day (E)9.5, and a fully septated heart is formed by about E13.5. Comparison of cardiomyocytes isolated from hearts at these two time points indicate that maturation of the mitochondria over this period is a crucial determinant of cardiomyocyte phenotype and function. Mitochondria exist as a complex dynamic filamentous network that is maintained by the equilibrium of two opposing pathways: mitochondrial fusion and mitochondrial fission. Disruption of the fusion process results in fragmentation of the mitochondria into rods or spheres, while elongated, interconnected filaments that typically cluster around the nucleus result from disruption of the fission process; reviewed in (Chen and Chan, 2005). These mitochondrial dynamics are tightly regulated in cardiac cell differentiation during development. Thus, at E9.5, the cardiomyocyte mitochondria are round, fragmented with few cristae and have an open mitochondrial permeability transition pore (mPTP). By contrast, at E13.5, the mitochondria are elongated and branched with more tubular cristae and a closed mPTP (Hom et al., 2011) (summarised in Fig. 1). Closure of the mPTP is believed to act to increase ATP generation rapidly through oxidative phosphorylation by increasing the coupling of the electron transport chain to ATP synthase (Weiss et al., 2003). The redox state of the cardiomyocyte appears to be a crucial determinant of this maturation transition. Thus, the levels of reactive oxygen species (ROS), which comprise the highly reactive, partial reductive products of molecular oxygen (Nathan and Cunningham-Bussel, 2013), appear much higher in the less differentiated cardiomyocytes, and crucially, treatment of these less differentiated cardiomyocytes with pro- or antioxidants either inhibited or promoted their differentiation, respectively (Hom et al., 2011). Consistent with these observations, H_2O_2 has been shown to induce mitochondrial fission in a dose-dependent and reversible fashion in a variety of cells; reviewed in (Bolisetty and Jaimes, 2013).

The process of early cardiogenesis from ES cells *in vitro* has also been widely studied (Boheler et al., 2002; Wobus et al., 2002). ES cells can be induced to differentiate *in vitro* into embryoid bodies (EBs) comprising many different cell types, including cardiomyocytes, which can be seen to beat in culture. The proportion of EBs displaying such beating cells is generally taken as a measure of cardiotypic differentiation. However, it should be noted that an increase in the proliferative capacity of the cardiac progenitor cells in this system would similarly give rise to an increase in the numbers of beating cells within EBs. Structurally, ES cells have a high nucleus:cytosol ratio, with scarce mitochondria that are small and immature with poorly developed cristae (Chung et al., 2007). By contrast, as ES cells terminally differentiate into cardiomyocytes, abundant mitochondria that have adopted a tubular structure and contain elongated cristae fill the perinuclear and interfibrillar spaces. This structural change is accompanied by decreases in the levels of expression of glycolytic enzymes and increases in the expression levels of enzymes involved in the electron transport chain, fatty acid β-oxidation and citric acid cycle. As a consequence, the cardiomyocytes display an elevated mitochondrial membrane potential, increased mitochondrial oxygen consumption and ATP production and reduced glycolysis (Chung et al., 2007). Thus the metabolic profiles of cardiomyocytes differentiating *in vitro* appear comparable to those undergoing cardiogenesis *in vivo*. The causal involvement of changes in cellular ROS and redox state in cardiomyocyte differentiation *in vitro* has also been demonstrated. The generation of pro- or antioxidant redox states within ES cells have, in general, acted to promote or inhibit cardiomyocyte differentiation *in vitro*, respectively (Sauer et al., 1999; Schmelter et al., 2006; Serena et al., 2009). This contrasts with the observation described above that mPTP closure, mitochondrial maturation and cardiomyocyte differentiation were inhibited by pro-oxidants and enhanced by antioxidants. Additionally, it suggests that the response to changes in cellular ROS may be dependent on the particular cellular stage of cardiogenic differentiation. This is further supported by observations that addition of H_2O_2 to differentiating ES cells can either enhance or impair the progression of the cardiogenic programme depending on the time of administration (Buggisch et al., 2007; Li et al., 2006; Puceat et al., 2003). The causal effects of changes in cellular redox on the metabolism of the differentiating cardiomyocytes and the precise cellular source(s) of this ROS remain to be elucidated. However, in *Xenopus laevis*, ROS production from the mitochondrial respiratory pathway has been suggested to be important for embryonic heart development (Chen et al., 2007), while we and others have demonstrated the importance of ROS derived from NADPH oxidase 4 (Nox4) in the very earliest stages of cardiogenesis *in vitro* (Li et al., 2006; Murray et al., 2013).

Metabolism in the foetal heart

In vivo, subsequent to the changes that occur within the mitochondria, and the closing of the mPTP described above (before E13.5 in the mouse), the foetal heart still maintains a highly glycolytic metabolic profile, with a very limited ability to carry out, in particular, fatty acid β-oxidation. Thus, the foetal heart produces most of its ATP production via glycolysis and oxidation of carbohydrates, particularly lactate (Lopaschuk and Jaswal, 2010). As the heart develops *in utero*, it must adapt to a series of changes, including a slowly increasing haemodynamic load as the peripheral vascular system develops (Patterson and Zhang, 2010). Over this