ELSEVIER

Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state

Alexandra N. Paradis[1], Maresha S. Gay[1] and Lubo Zhang

Center for Perinatal Biology, Division of Pharmacology, Department of Basic Sciences, Loma Linda University School of Medicine, Loma Linda, CA 92350, USA

Cardiomyocytes possess a unique ability to transition from mononucleate to the mature binucleate phenotype in late fetal development and around birth. Mononucleate cells are proliferative, whereas binucleate cells exit the cell cycle and no longer proliferate. This crucial period of terminal differentiation dictates cardiomyocyte endowment for life. Adverse early life events can influence development of the heart, affecting cardiomyocyte number and contributing to heart disease late in life. Although much is still unknown about the mechanisms underlying the binucleation process, many studies are focused on molecules involved in cell cycle regulation and cytokinesis as well as epigenetic modifications that can occur during this transition. Better understanding of these mechanisms could provide a basis for recovering the proliferative capacity of cardiomyocytes.

Introduction

Cardiomyocytes are the functional unit of the heart; therefore the number of viable myocytes dictates cardiac function. The total cardiomyocyte population is determined early in life during fetal development and around birth, with negligible increases thereafter [1]. Hence, preservation of cardiomyocyte number will fortify the heart and enable adequate response to stress later in life. It has long been understood that the heart loses proliferative capacity soon after birth in most mammals [2–4]. This timeframe is consistent with the conversion of cardiomyocytes from a mononucleate to binucleate phenotype. Binucleation is a characteristic of terminally differentiated cells that are unable to proliferate, whereas mononucleate cells continue to cycle. Early in normal fetal development the majority of cardiomyocytes are mononucleate, allowing growth to be achieved by proliferation. In the timeframe surrounding birth, heart maturation occurs where mononucleate cells begin the transition to a binucleate phenotype. The uncoupling of cytokinesis from karyokinesis and ultimate exit of the cell cycle characterize the transition, resulting in binucleation [5]. Subsequent increases in heart size are independent of proliferation and the result of increases in individual cell size termed hypertrophy.

In humans, the fetal heart consists of mainly mononucleate cardiomyocytes and this is therefore the time at which most proliferation occurs. Just before birth, binucleation begins and can extend into early neonatal life. Similarly, sheep follow this pattern of development, providing a close model for studying the heart. Rodents are another commonly used model however it is to be noted that cardiomyocyte binucleation in rodents begins and ends within the first two weeks after birth [5]. In all these species, the adult heart contains the greatest amount of binucleate cells when compared with the fetal and neonatal stages. However the percentage of binucleate cells within the adult heart varies among species, as reviewed by Botting *et al.* [6]. In humans, there is considerable debate about the amount of binucleate cells present in the adult heart, with values ranging from 25 to 60% [6]. Rodents and sheep, by contrast, have approximately 90% of the cardiomyocyte population binucleated [6].

The physiological importance of binucleation is still poorly understood. A plausible explanation is that multinucleation optimizes cellular response, enhancing cell survival when coping with stress [7]. Another argument is that binucleation occurs to meet the high metabolic demand of cardiomyocytes. As such, binucleation has an advantageous role in enabling the cell to generate twice the amount of RNA to synthesize proteins [3]. This review discusses factors involved in cardiomyocyte transition including

E-mail address: lzhang@llu.edu.

[1] These authors contributed equally to this work.

1359-6446/06/$ - see front matter http://dx.doi.org/10.1016/j.drudis.2013.10.019

Please cite this article in press as: A.N. Paradis, et al., Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state, Drug Discov Today (2013), http://dx.doi.org/10.1016/j.drudis.2013.10.019

alterations to its timing, the underlying molecular mechanisms and the role of epigenetic regulation and therapeutic targets.

Premature transition

The intrauterine environment is highly influential on the health of an individual. Its influence can lead to structural and functional adaptations of several organs, including the heart. Persistence of these adaptations can increase vulnerability later in life to diseases including metabolic syndrome and cardiovascular disease [6,8,9]. Altered cardiomyocyte number could be responsible for this increased susceptibility. In support, animal studies provide evidence that fetal stress caused by hypoxia [10], glucocorticoids [11] or maternal malnutrition [12,13] affects the number of cardiomyocytes and the ability of the heart to cope with stress later in life.

Hypoxia

Hypoxia is a major fetal stressor induced under a variety of conditions including nicotine exposure, high altitude pregnancy, preeclampsia and placental insufficiency. The long-term implications of this adverse environment have been well established [8,14]. Recent studies have shown that hypoxia directly reduces proliferation in fetal rat cardiomyocytes [15]. In other studies maternal hypoxia was found to result in increased size and percent of binucleate cardiomyocytes [10] along with remodeling of the fetal and neonatal rat heart [16]. Fetal sheep anemia studies by Jonker *et al*. also reported larger and more mature cardiomyocytes [17]. This was marked by increases in the amount of binucleate cardiomyocytes in the right ventricle.

By contrast, studies of hypoxia-inducible factor 1α (HIF1α) overexpressed in transgenic mice reveal a possible role in improving cardiac function and reducing infarct size after myocardial infarction (MI) [18]. Moreover, C3orf58, a hypoxia- and Akt-induced stem cell factor (HASF) has been shown to have a positive effect on proliferation of cardiomyocytes in rodents [19]. Similarly, hypoxia was shown to have a role in increasing proliferation of adult zebrafish cardiomyocytes, *in vitro* studies reveal this can be achieved by hypoxia-induced dedifferentiation [20]. These studies indicate a possible dual role of hypoxia in regulating cardiomyocyte proliferation. Altogether demonstrating that hypoxia is involved in cardiac remodeling and can directly affect cardiomyocyte endowment of the heart.

Glucocorticoids

Glucocorticoids are a class of hormones essential to normal lung development and the regulation of the cardiovascular system. Although glucocorticoids are essential to the development and survival of the fetus, excessive exposure has negative implications including delayed maturation of astrocytes [21], reduced birth weights [22] and altered glucocorticoid receptor expression [23].

Evidence exists for a role of glucocorticoids in regulating cardiomyocyte development. Early studies by Rudolph *et al.* reported a reduction in cardiomyocyte proliferation after fetal sheep cortisol infusion, associated with hypertrophic growth [24]. However, more recently, studies in fetal sheep revealed increased proliferation without an increase in cardiomyocyte size after cortisol infusion [11]. In this latter study no differences in length, width and overall percentage binucleation of cardiomyocytes were observed between cortisol-treated and nontreated groups. In addition, the cortisol treatment did not drive the maturation of cardiomyocytes but rather stimulated their entry into the cell cycle suggesting cortisol is associated with hyperplastic growth. These opposing results are probably the result of the different methods of quantification used by the researchers; and are further discussed by Giraud *et al.* [11].

In the fetal rat low-dose dexamethasone, a synthetic glucocorticoid, was found to decrease fetal bodyweight when administered prenatally by Torres *et al.* [25]. In this study the dexamethasone-treated group was found to have increased cardiomyocyte proliferation in the fetal heart as compared with control. In addition, a sex-dependent component of cardiomyocyte proliferation was observed, with females having significantly more DNA synthesis compared with males. Taken together, these findings provide evidence for premature glucocorticoid exposure associated with a developmental delay of heart maturation.

In neonates dexamethasone treatment has been found to decrease total cardiomyocyte number [26] possibly by decreasing proliferation [26,27]. De Vries *et al.* [26] reported reduced proliferation during neonatal day 2–4 with no subsequent changes. This study also noted no apoptosis, supporting suppressed proliferation as the cause of lower cardiomyocyte number. These reductions in cardiomyocyte number were noted to continue into adulthood, associated with reduced systolic function [28]. It is evident that the effect of glucocorticoid treatment is dependent on the time of exposure. Fetal exposure results in increased cardiomyocyte proliferation, whereas neonatal exposure has an opposite effect. This provides evidence for a time-dependent mechanism of glucocorticoid action, highlighting the importance of monitoring perinatal circulating glucocorticoid levels because of the diverse impact on heart development.

Hypertension

The heart must constantly adapt to the hemodynamic load of the body. In fetal sheep acute hypertension has been found to increase cardiomyocyte proliferation, followed by increases in cell size, whereas longer-term hypertension leads to an increase in binucleate cells [29]. Jonker *et al.* induced fetal hypertension in sheep by intravascular plasma infusion for 4 or 8 days. In both hypertensive groups the heart weight was increased, and the number of mononucleate cardiomyocytes was more than double that of control. In addition, at both time points a significant amount of myocytes was found to be actively cycling. However, after 8 days of infusion the amount of binucleation in the left and right ventricle was significantly elevated. Thus, this suggests there are two physiological responses to hypertension in the fetal heart. Initially hypertension (measured after 4 days) stimulated cell cycle activity, and with extended hypertension treatment (8 days) a marked increase in binucleation of the heart is noted. The initial increase in mononucleate cells could represent a short-term change whereas the later binucleation could reflect long-term premature loss of proliferation [29].

Post-mortem studies of the adult human heart revealed increased ploidy and binucleation after cardiac injury [30]. It is believed that the loss of cardiomyocytes in such pathological conditions increases the hemodynamic load on the remaining cardiomyocytes. In this way the increased workload could stimulate the heart to proliferate

Please cite this article in press as: A.N. Paradis, et al., Binucleation of cardiomyocytes: the transition from a proliferative to a terminally differentiated state, Drug Discov Today (2013), http://dx.doi.org/10.1016/j.drudis.2013.10.019