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Polyploidy and nuclear phenotype characteristics of cardiomyocytes from diabetic adult and normoglycemic aged mice

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ABSTRACT

The frequency of polyploid nuclei in the aging human heart is in sharp contrast with that in the human liver. An inverse pattern exists between the mouse heart and liver cells. Ploidy degrees in mouse hepatocytes under hyperglycemic conditions are elevated to higher levels than those in aged hepatocytes. In this study, image analysis cytometry was used to investigate the effect of diabetes and aging on Feulgen-DNA quantities, ploidy degrees, nuclear shapes and chromatin texture in mouse cardiomyocytes compared to previously reported data for mouse hepatocytes. Adult, non-obese diabetic (NOD) hyperglycemic and normoglycemic females and 56-week-old normoglycemic BALB/c females were used. A small percentage (~7%) of the cardiomyocyte nuclei in severely hyperglycemic NOD adult mice possessed higher ploidy values than those in the 8-week-old normoglycemic mice. Surprisingly, the Feulgen-DNA values and the frequency of nuclei belonging to the 4C and 8C ploidy classes were even higher (~6%) in normoglycemic NOD specimens than in age-matched hyperglycemic NOD specimens. Additionally, a pronounced elongated nuclear shape was observed especially in adult normoglycemic NOD mice. In conclusion, NOD mice, irrespective of their glycemic level, exhibit a moderate increase in ploidy degrees within cardiomyocyte nuclei during the adult lifetime. As expected, aging did not affect the Feulgen-DNA values and the ploidy degrees of cardiomyocytes in BALB/c mice. The differences in ploidy degrees and chromatin textures such as absorbance variability and entropy, between adult NOD and aged BALB/c mice are consistent with other reports, indicating dissimilarities in chromatin functions between diabetes and aging.

1. Introduction

Polyploid nuclei occur at a high frequency in the human heart with advancing age, unlike in the human liver. In human adults, most left ventricular cardiomyocyte nuclei are affected by polyploidization (33%, diploid nuclei, 56% tetraploid nuclei, and ~11%, octoploid nuclei) (Bergmann et al., 2011). An inverse pattern has been observed when comparing the heart and liver cells in mice (Anatskaya and Vinogradov, 2007, 2010). Mouse cardiomyocytes have predominantly diploid nuclei (Anatskaya and Vinogradov, 2007); a maximum of 13% of mouse cardiomyocyte nuclei may become tetraploid (Alkass et al., 2015).

Hepatocytes from hyperglycemic adult, female, non-obese diabetic (NOD) mice show elevated levels of nuclear ploidy degrees compared to those from normoglycemic BALB/c aged mice (Mello et al., 2009; Ghiraldini et al., 2012). The increased polyploidy in hepatocytes from hyperglycemic NOD mice may represent an attempt to elicit a protective response or a compensatory mechanism against diabetic stress (Ghiraldini et al., 2013).

NOD mice are a valuable experimental model for type-1 diabetes research. They provide a reductionist system for studying the development of a chronic autoimmune process against pancreatic beta cells, which involve different types of immune cells that participate in complex multicellular interactions (Yang and Santamaria, 2003). The NOD strain is a polygenic model for autoimmune type-1 diabetes that is characterized by hyperglycemia and insulitis, a leukocyte infiltrate of the pancreatic islets that leads to the selective destruction of beta cells (Yang and Santamaria, 2003; The Jackson Laboratory, 2017). In NOD mice, there is a combination of apparently normal alleles at numerous loci associated with diabetes (Yang and Santamaria, 2003). In the NOD mouse model, diabetes develops spontaneously, with genetics and pathological outcomes that have many similarities to those of human type-1 diabetes (Jayasimhan et al., 2014; Boldison and Wong, 2016; Pearson et al., 2016). This mouse model is a tool for investigators to evaluate the pathogenesis of type-1 diabetes-induced complications (Shi et al., 2013) and it has provided an invaluable understanding of basic immune pathogenesis, genetic and environmental risk factors, and immune-targeting strategies (Pearson et al., 2016). Because the

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Table 1
Feulgen-DNA C-classes of cardiomyocytes of normoglycemic and hyperglycemic mice.

Cells	Mouse age (weeks)	Mouse lineage	Glycemic state	No. of animals	Relative frequencies (%) of Feulgen-DNA C-classes			No. of nuclei
					2C	4C	8C	
Lymphocytes*	8	BALB/c	Normoglycemia	3	100.	–	–	90
Cardiomyocytes	8	BALB/c	Normoglycemia	4	90.34	9.66	–	300
		NOD/Uni	Normoglycemia	4	93.00	7.00	–	300
	19–29	NOD/Uni	Hyperglycemia	8	85.99	13.64	0.37	800
		NOD/Uni	Normoglycemia	7	79.69	18.31	2.00	699
		BALB/c	Normoglycemia	5	93.39	6.61	–	499
	56	BALB/c	Normoglycemia	5	94.20	5.80	–	500

* 2C control.

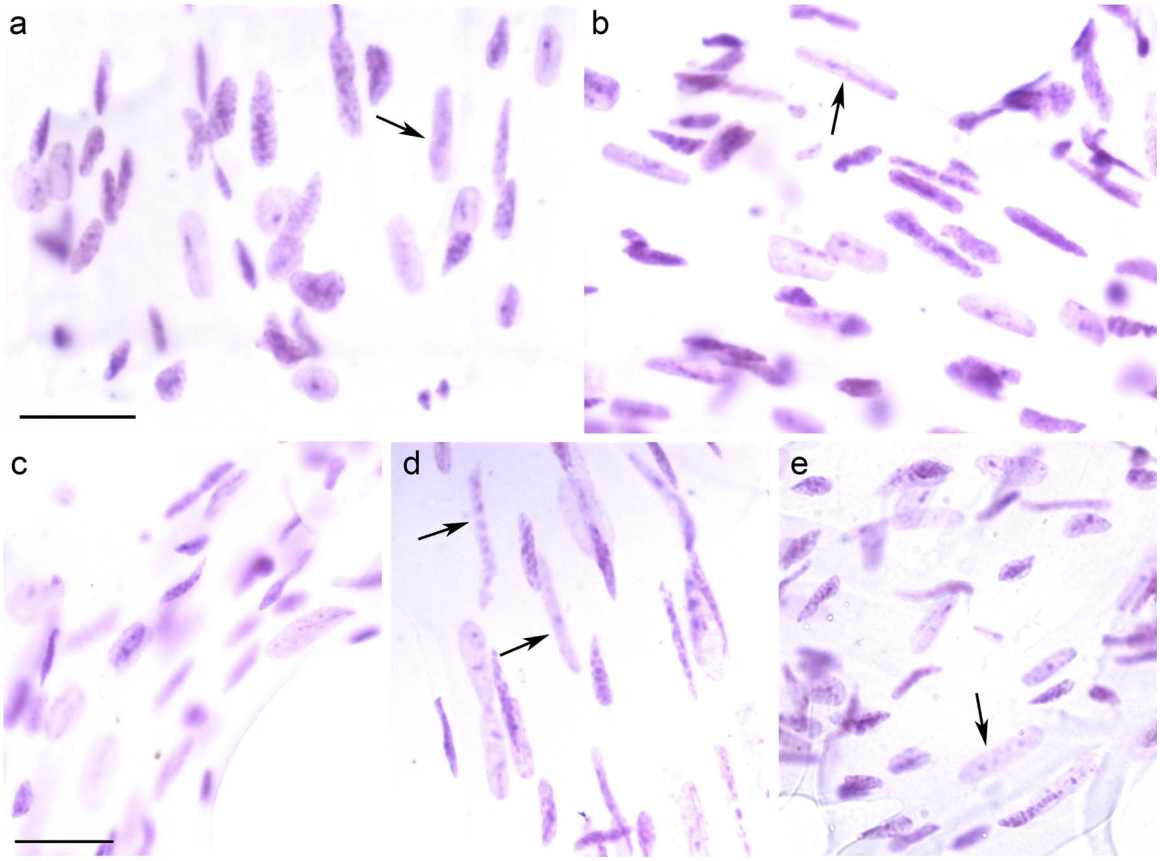

Fig. 1. Light microscopy view of nuclear phenotype variability in Feulgen-stained mouse cardiomyocytes. a. Normoglycemic 8-week-old BALB/c mouse; b. Normoglycemic 8-week-old NOD mouse; c. Normoglycemic aged BALB/c mouse; d. Normoglycemic adult NOD mouse; e. Hyperglycemic adult NOD mouse. The arrows indicate nuclei with a small Feret ratio. The bars represent 20 μm.

incidence of spontaneous diabetes mellitus in NOD mice is 60–80% in females and 20–30% in males and because the onset of this disease is delayed in males by several weeks (Makino et al., 1980), NOD females have been more widely used in diabetes studies.

In human and animal models of diabetes, several functional and structural alterations, including diastolic dysfunction and/or ventricular systolic dysfunction, as well as cardiac hypertrophy, are known to affect the heart (Shiomi et al., 2003; Hayat et al., 2004). Other altered characteristics include cardiomyocyte stiffness, interstitial collagen deposition, evidence of poly(ADP-ribose) polymerase activation, reduction of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2a) pump expression levels, changes in F-actin organization and stress-induced apoptotic cell death (Cai et al., 2002; Pacher et al., 2002; Zou and Xie, 2013; Benech et al., 2014; Guo et al., 2015). The levels of SIRT1, an NAD^+-dependent nuclear deacetylase, in heart cells from diabetic rats, are decreased and are restored only after implementing treatments that attenuate endoplasmic reticulum stress and caspase-12 activation (Guo et al., 2015), which contrasts with hepatocytes from diabetic mice, in which SIRT1 production increases (Ghiraldini et al., 2013). In NOD mice, the Na pump activity is depressed in the heart as well as in the kidney and pancreas, correlating with the progression of elevated blood glucose concentration (Shi et al., 2013). Cardiovascular autonomic dysfunction has also been demonstrated in NOD mice with plasma glucose levels > 300 mg/dL (Moraes et al., 2013).

Until now, differing from what has been reported for mouse hepatocytes, there are no studies that investigated whether nuclear ploidy degrees in mouse heart cells are affected by type-1 diabetes. Polyploidization that occurs through incomplete cytokinesis may protect cells against genotoxic damage by increasing gene copy numbers, although this process may also lead to chromosomal instability (Pandit et al., 2013). Regarding whether nuclear phenotypes in cardiomyocytes are affected by type-1 diabetes, the presence of irregularly sized cell

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