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Neonatal cardiomyocyte ploidy reveals critical windows of heart development

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Abstract

Background: The aim of our study was to find out, whether cardiomyocyte genome duplication participates in developmental programming of adult hypertension and impaired heart aerobic capacity, and if it does, whether ploidy-related programming is reversible and what are the timeframes of the most critical window. For this propose we studied the effect of the well-known factors of programming, including growth retardation, infection, and cardiac overload on the level of neonatal cardiomyocyte ploidy, protein content and shape.
Methods: Using the model of rat cryptosporidial gastroenteritis, we shifted the time point of infection day by day through the neonatal period and traced the immediate and postponed effects of disease on isolated cardiomyocyte ploidy, phenotype, and protein content.
Results: We found that gastroenteritis caused cardiac atrophy and a burst-like premature genome accumulation, elongation, narrowing and protein loss in the cardiomyocytes. These changes resulted in sharp increase of DNA content at the expense of contractile proteins. We also revealed clear indications of critical window of heart development during the peak of cardiomyocyte transition from proliferation to hypertrophy. After the rehabilitation, the atrophy of heart and cardiomyocyte remodelling showed a conspicuous restoration, whereas the hyperpolyploidization did not regress. An irreversible manner of excessive genome duplication and its well-known ability to alter gene expression confirm our suggestion that it is implicated in the ontogenetic programming of heart development.
Conclusion: We provided the first evidence that developmental programming can operate through cardiomyocyte genome duplication and that the critical window coincides with cell transition from proliferation to hypertrophy. Our data help determine the timing of critical window for human heart and would allow successful prevention of human cardiac abnormalities even before they become tangible.

Keywords: Quantitative remodeling; Developmental programming; Polyploidy; Gastroenteritis; Animal model of human disease

1. Introduction

Epidemiological studies evidence that many adult diseases have their roots in early ontogeny. This phenomenon was named 'developmental programming' [1]. The heart is the organ that is most susceptible to this programming: elevated blood pressure, impaired heart aerobic capacity, increased risk for ischemia and stroke are not considered as purely 'adult' problems anymore because they have roots in the prenatal and early postnatal development [2,3].

The problem of neonatal programming attracts much attention [4–6]. A great progress has been made in the understanding of how environmental factors modify the cardiovascular development. The role of growth retardation, suckling quality, maternal care, chronic hypoxia and anemia, glucocorticoid external influx and inflammatory infectious diseases was defined [2,4,7]. The timeframe of programming has been shown to cover the fetal and neonatal periods in rodents and the fetality, infancy, childhood and even puberty in humans [8].

At the same time, notwithstanding the numerous studies examining tens of thousands of human subjects and many

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animal models, the link between early life and adult cardiac diseases is poorly understood [5]. Why the heart is so sensitive? What are the exact mechanisms of programming? Whether different windows possess different mediators of permanent changes? When programming operates most dramatically? These questions are still opened.

We suggest that postnatal life-long epigenetic changes may stem from the irreversible cardiomyocyte polyploidization and this phenomenon may be involved in the ontogenetic programming of adult cardiac abnormalities and low aerobic capacity. If this is true, the critical developmental window should coincide with the time of cardiomyocyte transition from proliferation to hypertrophy when the polyploidization takes place. It is well known that polyploidization changes the surface-to-volume ratio of the nucleus. Doubling the genome is expected to double the volume that is occupied by chromatin, but cause only a 1.6-fold increase in the nuclear envelope surface and thus might cause a gene dosage imbalance and corresponding regulatory repercussions [9]. The recent data confirm that even the subtle changes of DNA spatial organization might dramatically affect gene promoter activity [10]. The recent microarray data obtained with organisms from different kingdoms showed the non-additive effects of gene products after polyploidization [11].

It was shown on the yeast model that polyploidy activates genes responsive to DNA damage and homologous recombination and suppress transcripts implicated in cytoskeleton, proliferation and mitotic spindle [12–14]. In mammalian hepatocytes and polyploidy showed similar effects: genes involved in DNA double-strand break repair and homologous recombination (specifically, HMG and RAD-family genes) and stress response increased their activity, whereas genes participating in aerobic respiration, cytoskeleton and mitotic spindle and immunity became less active [15,16]. In vascular smooth muscle cells genome duplication decreases expression of genes involved in inflammation [17].

Cardiomyocytes undergo polyploidization in the early postnatal life when they switch from proliferation to hypertrophy [18]. Compared with other mammals, human possess cardiomyocytes with the highest ability to multiply genomes [19]. At the same time, healthy adults differ dramatically by cardiomyocyte ploidy. Some people live with the almost completely octaploid hearts, whereas hearts of others (of the same age, mass and gender) contain predominantly tetraploid myocytes [20]. The significance and genesis of this variability are unknown.

Clinical studies report that genome duplication is higher in hearts with impaired cardiac function caused by pathologic hypertrophy, atrophy and congenital diseases. In the diseased hearts, the fraction of highly polyploid cardiomyocytes with 16, 32 and even 64 genomes usually exceed 30% and cells with 128 genomes can present in tangible amount [21–23]. The impairment of cardiac function may stem from the decrease of myofibril energy supply and protein content per genome [23–25].

To find out, whether genome duplication may be involved in developmental programming, we should answer the questions whether the well-known factors of programming, such as growth retardation, infection, and cardiac overload affect the level of neonatal cardiomyocyte ploidy, protein content and shape. If they do, whether these effects are reversible and what are the timeframes of critical ontogenetic periods?

For this proposes, we developed a postnatal suckling-weanling rat model of experimental gastroenteritis challenged by a worldwide protozoan pathogen *Cryptosporidium parvum*. This disease is of particular danger for children because it like a double-edged sword strikes them with growth retardation, inflammation and premature cardiac overload simultaneously [2,26]. Growth retardation accompanies malnutrition caused by gastroenteritis. Heart overload originates from severe cardiac arrhythmias, reduction of cardiomyocyte myofibril protein synthesis and hormonal misbalance [27].

We examined sensitivity of cardiomyocyte ploidy to cardiac overload through the neonatal period. We used the remodeling of cell shape as indicator of moment when ploidy became resistant to overload (thus indicating the end of the critical time window). Cardiomyocyte and heart shapes are very plastic and react readily to overload through entire life [28,29]. Therefore it continues to response to overload even when ploidy level looses its sensitivity.

Taken together, our data support the link between the immediate adverse effects caused by neonatal gastroenteritis and the long-term cardiac atrophy (indicative of the decrease of aerobic capacity), cardiomyocyte remodeling, protein loss, irreversible hyperpolyploidization and elevated DNA to protein content ratio. We also obtained clear indications that the time of cardiomyocyte polyploidization coincides with the critical window of heart development.

2. Materials and methods

2.1. Experimental design

To find out whether polypoidy is implicated in neonatal programming of cardiac aerobic capacity and to determine the critical developmental window of heart, we designed a new model of experimental cryptosporidial gastroenteritis. The model of cryptosporidial gastroenteritis matches our aim well because it has clearly defined time of complete cessation and lasts only four days. These features allow us evaluate immediate and postponed effects of the disease with high accuracy. Sliding the moment of infection from day 4 to day 18, we examined heart sensitivity through complete neonatal period. We evaluated heart mass to body mass ratio, cardiomyocyte ploidy, protein amount and geometry. Heart mass to body mass ratio (relative heart mass) reflected heart aerobic capacity; cardiomyocyte ploidy, protein amount and geometry indicated cardiac pathologic overload. We performed the immediate study at day 5 after infection, the short-term study after two weeks of restoration, and the long-term study after a month of restoration.