Ageing of the heart reversed by youthful systemic factors!

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The EMBO Journal (2013) 32, 2189–2190. doi:10.1038/emboj.2013.162; Published online 16 July 2013

Age-associated changes in tissue maintenance and repair have severe consequences to human physiology. The signals and mechanisms that cause age-related tissue demise are unclear. A recently published study in *Cell* (Loffredo *et al*, 2013) proposes that blood-borne factors in the adult systemic environment are lost during ageing, which leads to cardiac hypertrophy. One such factor is GDF11. Exposure of aged mice to youthful systemic factors or GDF11 decreases cardiac hypertrophy of the heart.

The ageing process is associated with a loss of tissue function through disrupted homeostatic and regenerative mechanisms (Liu and Rando, 2011). Throughout the life of any organism, the cells of the body experience various extrinsic and intrinsic changes that ultimately impact tissue function. The relative contribution of extrinsic factors and intrinsic modifications that can impact any cell is likely to be dependent on the function of the cell, its turnover throughout life and the environment in which it resides.

The regenerative decline of many tissues observed during ageing is thought to be due to stem cell demise, regulated in part through changes in the composition of blood-borne factors present in the systemic environment. Parabiosis is an experimental paradigm used to study the role of blood-borne factors in many cellular processes (Finerty, 1952). In this technique, two mice are surgically joined and develop a shared blood circulation; therefore, the tissues of one mouse are exposed to its partner's circulatory factors. Parabiosis studies involving adult and aged mice have revealed the presence of stimulatory and repressive blood-borne factors in the systemic environment that impact stem and progenitor cell function in response to injury (Conboy *et al*, 2005; Brack *et al*, 2007; Villeda *et al*, 2011; Ruckh *et al*, 2012). However, tissues that do not rely on stem cells also undergo age-dependent decline. For example, the cardiac muscle undergoes ventricular hypertrophy during ageing, often leading to diastolic heart failure due to the increased size of individual differentiated cardiac myocytes. Is this also regulated by the systemic environment and, if so, how?

In their present work, Loffredo *et al* (2013) have tested the hypothesis that age-dependent changes in systemic factors promote cardiac hypertrophy. The authors report an age-dependent increase in cardiac myocyte size that is coupled to increased weight of the heart muscle. Remarkably, 4 weeks of parabiosis led to a significant reversion of age-induced

cardiac hypertrophy. Importantly, these effects were gender-independent and did not arise from the parabiosis technique itself, or changes in blood pressure. The authors identified that the myocyte cross-sectional area was decreased in aged mice paired with adult mice, and thus blood-borne factors were acting directly on the terminally differentiated cell. In comparison, analysis of adult mice that were paired with aged mice for up to 10 weeks did not show any change in the size of myocyte or weight of the heart. Together, these results are consistent with the loss of youthful factors in the aged systemic milieu that repress myocyte size rather than the accumulation of hypertrophic factors during ageing. Loffredo *et al* (2013) also investigated the molecular nature of cardiac hypertrophy, using a few molecular markers of cardiac hypertrophy, such as brain natriuretic peptide (BNP) and atrial brain natriuretic peptide (ANP). From this result, the authors claim that circulatory factors can reverse some molecular aspects of cardiac ageing.

Identification of the blood-borne factors that impact ageing is of obvious significance. The authors used an aptamer-based proteomic platform to hunt for the 'fountain of youth.' Aptamers are chemically modified nucleotides that

Figure 1 In adult mice, the presence of GDF11 in the systemic environment acts to restrain cardiac myocyte size. Loss of blood-borne GDF11 in aged mice drives cardiac myocyte hypertrophy. Exposure of aged mice to youthful systemic factors through parabiosis or injection of GDF11 reverses morphological and molecular markers (ANP and BNP) of age-induced cardiac hypertrophy.

act as highly specific protein binding reagents. They can be multiplexed and transformed into a quantifiable readout using a hybridization array. Using this method and by validating using western blots, the authors show that levels of growth differentiation factor 11 (GDF11), a TGF β superfamily member, were consistently lower in aged compared to adult plasma.

To test whether GDF11 was sufficient to reverse age-induced cardiac hypertrophy, recombinant GDF11 was delivered daily via intraperitoneal injection for 30 days to aged mice. GDF11 administration led to a significant decline in weight and left ventricular cross-sectional area of the aged heart, albeit not a complete reversion to that of an adult heart. That GDF11 did not achieve complete revision of hypertrophy may be due to technical reasons, such as non-uniform access of the injected protein to the cardiomyocytes, or biological reasons, such as non-overlapping signalling pathways that control cardiomyocyte size. Nevertheless, the fact that single growth factors can be injected into the blood stream to substantially decrease age-dependent cardiac hypertrophy is a tantalizing prospect for the treatment of human cardiac hypertrophy (Figure 1).

To test the utility of GDF11 treatment in reversing other models of cardiac hypertrophy, two different approaches were tested: (1) phenylephrine-induced hypertrophy of neonatal cardiomyocytes *in vitro* and (2) pressure overload, via aortic constriction of adult mouse heart. These two assays provided contrasting results; phenylephrine-induced hypertrophy as measured by protein synthesis rate (3H leucine incorporation) was prevented by prior incubation with GDF11 and its TGF β superfamily member, Myostatin, whereas pressure overload hypertrophy was not mitigated by GDF11 treatment, administered after aortic constriction.

Therefore, ageing-induced cardiac hypertrophy may have a distinct causality (decreased levels of GDF11) compared to acute models of hypertrophy in adult mice. It is also possible that additional cooperatively acting signalling pathways participate in adult hypertrophy. This would act to minimize any effects of GDF11 augmentation in adult mice. In addition, there may be a specific time window relative to hypertrophic stimuli, owing to which GDF11 treatment elicits a more

favourable cellular outcome. The results presented by Loffredo *et al* (2013) illustrate the complexity of studying the mechanisms behind the disease pathology.

Seeking to identify the source of GDF11, the authors measured GDF11 levels across different tissues. Its expression was most abundant in the adult spleen and decreased during ageing. Interestingly, GDF11 was also observed at the intercalated discs of aged hearts. A couple of questions arise from these observations. Is the adult spleen the source of cardiac GDF11 and why is the GDF11 that is located in aged hearts insufficient to protect from hypertrophy? Answering these questions will be experimentally challenging but nonetheless important.

This report identifies a blood-borne factor that is abundantly expressed in adult mice and acts to repress cardiac hypertrophy. Circulatory factors have the potential to act on multiple cell types. The present study illustrates that systemic factors can influence the size of terminally differentiated cells. Does GDF11 regulate the cell size of other tissues? In contrast to the cardiac muscle, other tissues such as bone and skeletal muscle undergo atrophy during ageing; thus, it is unlikely that decreasing levels of GDF11 in the aged systemic environment function to increase the cell size of multiple tissues during ageing. However, there may be other positive roles of blood-borne GDF11 in younger mice. For example, the stimulatory effect of youthful systemic factors on tissue regeneration may involve GDF11.

A picture is beginning to emerge whereby multiple factors found in adult (GDF11) and aged (TGF β 2, Complement C1q and CCL11) serum can alter cell function (Villeda *et al*, 2011; Naito *et al*, 2012; Loffredo *et al*, 2013). In addition, it is apparent that the aged niche becomes deregulated, leading to a loss of stem cell homeostasis (Chakkalakal *et al*, 2012). Future work should focus on understanding how distinct factors from the systemic environment and the micro-environment integrate to dictate cellular outcome across different tissues during ageing.

Conflict of interest

The author declares that he has no conflict of interest.

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