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Telomere lengths and the rejuvenating factor GDF11 in coronary artery disease

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Background/Introduction: Telomeres are dynamic chromosome-end structures that protect DNA in our genome from deterioration. Throughout life, telomere lengths (TLs) become shorter, and the rate of this process has been associated with lifestyle and the onset of age-related diseases. Moreover, the aging-associated factor growth differentiation factor 11 (GDF11) has been suggested as a rejuvenating factor. Together, the interplay of these aspects that possibly modulate the aging process is of interest to explore.

Purpose: To investigate the associations between leukocyte TLs (LTLs) and chronological age, comorbidities and clinical outcome in patients with coronary artery disease (CAD). Potential covariations between LTL and GDF11 were further assessed.

Methods: 300 patients with stable CAD, 20% female, age range 36-81 yrs, were included. Within 2 years, a composite of new clinical events (unstable angina pectoris, myocardial infarction (MI), stroke and deaths) was recorded. DNA and RNA were isolated from whole blood for the analysis of LTLs and GDF11 gene expression, respectively. LTLs were measured by PCR using telomere-specific primers and determined relatively to a single copy gene (36B4). The amount of GDF11 was measured as relative quantification using PCR and the house-keeping gene β2M.

Results: We observed that patients with previously suffered MI presented with 20% shorter LTLs vs. patients without ($p = 0.015$), however, only in men ($p = 0.009$, $n = 115$). To further explore this association, LTLs were divided into quartiles (Qs) and further dichotomized at the distinctive cut-off level between Q3 and Q4. LTLs in the upper Q were associated with 60% lower frequency of having suffered a previous MI ($p = 0.008$, adjusted for age, treated hypertension, body mass index (BMI), triglycerides, HDL and fasting glucose). LTLs were not differently distributed according to sex or the presence of treated hypertension (52%), diabetes type 2 (25%), metabolic syndrome (35%) and clinical outcome (12%). In the total cohort, LTLs were further inversely correlated to age ($r = -0.17$, $p = 0.007$), however, only in women ($r = -0.37$, $p = 0.006$). In all subjects, GDF11 gene-expression was weakly and inversely correlated to age ($r = -0.16$, $p = 0.010$). Overall, no correlation was observed between LTLs and gene-expression of GDF11. However, when dividing into subgroups, a strong correlation was observed in overweight women ($BMI > 25$), ($r = 0.40$, $p = 0.028$).

Conclusions: Although no association between LTLs and clinical outcome was observed, LTLs correlated to the severity of CAD in men, in this case previously suffered MI. In women, LTLs seem to be more associated with age. The results may indicate gender-related differences in regulatory mechanisms of TLs and a possible metabolically influenced association between LTLs and GDF11.

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Duchenne muscular dystrophy leads to compromised genomic stability in stem cells and depletion of cardiac progenitors in failing heart

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Duchenne muscular dystrophy (DMD) is a genetic condition characterized by the lack of functional dystrophin. The progressive muscular pathology has devastating effect on the quality and lifespan of male patients. Majority of the DMD patients develops heart muscle fibrosis and cardiomyopathy, eventually leading to heart failure and premature death. Although several molecular mechanisms leading to the DMD cardiomyocyte death were described during the recent decades, the link between dystrophin deficiency and delayed onset of cardiomyopathy is still unclear. Recent evidence suggests involvement of progenitor population failure: thus we focused on studying DMD stem cells.

First we used mdx mouse model lacking dystrophin, to analyze c-kit+ cardiac progenitor cells (CPCs) depletion and its mechanism. We observed dramatic increase in CPCs population in young adult (2-3 months) mdx mice hearts, followed by steep decrease in mature mdx adult (5-6 months) animals, which is in contrast to WT mouse hearts, where the CPCs population size in both young and mature hearts is stable. The analysis of double strand breaks demonstrated that CPCs depletion in mdx animal hearts is associated with elevated nuclear DNA damage.

In order to dissect the mechanism of CPCs depletion in humans, we used DMD patient specific induced pluripotent stem cell model and human embryonic stem cells with dystrophin mutation introduced by CRISPR/Cas technology (DMD hPSC for both models). We observed that absence of dystrophin in DMD hPSC leads to dysregulation of nitric oxide synthase (NOS) activity, resulting in significantly elevated reactive oxygen species (ROS). Inhibition of NOS activity results in lowering ROS level in DMD hPSC. Elevated ROS level in DMD hPSC was further associated with increased DNA damage. Both the inhibition of NOS, as well as ROS scavenging, results in DNA damage reduction. Further, we observed that DMD phenotype is associated with increased mutation frequency of the hPSC, which suggests causal link leading from dystrophin deficiency, via NOS dysregulation, to the elevated ROS and subsequent DNA damage. Finally, we showed compromised genomic stability of DMD stem cells by analysis of mutant frequency.

Based on these results, we hypothesize that dystrophin deficiency leads to elevated proliferation of CPCs, presumably by cardiomyocyte damage/death or inflammatory response. We suggest that elevated proliferation together with NOS induced-ROS mediated-genomic instability leads to CPCs depletion, and subsequently to limited regenerative capacity of the heart muscle. In contrast to plain cardiomyocyte damage, this chain of events could explain the delayed onset of cardiac symptoms.

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Hypoxia activates stem cells in myocardial tissue

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Background: Regeneration of the myocardium after ischemic damage is an actual problem of current regenerative medicine. Endogenous stem cells of the heart have been described. These cells retain their regenerative potential in the postnatal period. Cardiac stem cells are a heterogeneous group of cells. Populations of these cells have been identified in the atria, ventricles, epicardium or pericardium of the heart. The discovery of these cells is an important step towards regenerative medicine, but the mechanism of activation of cardiac stem cells remains little known.

Purpose: Our aim was to compare cardiac stem cells from peri-infarction area to cardiac stem cells from healthy area of the myocardium in the experimental model of acute hypoxic damage. We compared cells by functional properties, such as growth rate, migration and differentiation abilities.

Methods: Myocardial infarction of the left ventricle was induced in Wistar rats by a permanent ligation of the left coronary artery. After 3 days cardiac stem cells were isolated from the ischemic area by enzymatic dissociation of the tissue healthy myocardial tissue was used as control. The cells were immunophenotyped by flow cytometric analysis. Proliferation rate was evaluated using growth curve method. Migration rate was estimated using scratch method. The cells were

differentiated in cardiogenic, adipogenic and osteogenic directions by addition of specific inducers to the cell culture medium. Differentiation efficiency was estimated by qPCR for specific markers of differentiation. Differentiated cells were stained for the specific markers by immunocytochemistry.
Results: Peri-infarct cells possessed a higher proliferative potential than the cells from healthy area of the myocardium. They also had a greater propensity to migrate to the area of a "scratch". According to a PCR experiment, the level of activation of specific differentiation markers (TNNT2, BMP2, Runx2 and Fabp4) was higher in peri-infarct cells.
Conclusion: Ischemic damage of the myocardium leads to activation of the internal regenerative potential of cardiac stem cells in vivo, which is most pronounced in the peri-infarction zone.

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Up-regulation of myocardial connexin-43 is involved in compensatory response of the heart to acute injury

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Rationale: Intercellular coupling at gap junction connexin (Cx) channels plays a fundamental role in mediating communication for establishment and maintenance of coordinated heart function as well as adaptive functions of differentiated cardiomyocytes. It has been shown that cardiac muscle exhibits innate cellular 'self-defense' from injury, whereby Cx43 can play a role in this process.
Purpose: We aimed to explore responses of myocardial Cx43 as well as PKCε, which phosphorylates Cx43, and MMP-2, which affect intercellular coupling, to acute cardiac injury induced by two distinct sub-lethal interventions in Wistar rats.
Methods: Male adult animals underwent either single chest irradiation at dose of 25 Gy or subcutaneous injection of isoproterenol (ISO, 120 mg/kg) and were compared with untreated controls. Forty-two days post-interventions, the hearts were excised and left ventricles were used for target protein analysis. Another part of excised hearts was perfused by Langendorff mode to assess heart function and susceptibility to ischemia or malignant arrhythmias.
Findings showed an increase of total as well as functional phosphorylated forms of myocardial Cx43 regardless the type of interventions. Enhanced phosphorylation of Cx43 coincided with increased PKCε expression in both models. Elevation of Cx43 was associated with its enhanced distribution on lateral surfaces of the cardiomyocytes in response to both interventions. In addition, focal areas of fibrosis along with miss-localization of Cx43 were found in post-ISO but not post-irradiated rat hearts. In parallel, MMP-2 activity was decreased in former while increased in the latter. Cardiac function was maintained and the susceptibility of the hearts to ischemia or malignant arrhythmias was not deteriorated forty-two days after sub-lethal interventions when compared to controls.
Conclusion: Altogether findings indicate that up-regulation of myocardial Cx43 is most likely involved in potentially salutary responses to acute heart injury. It appears that triggering of self-defense machinery can be expected in response to acute cardiac stress or injury.

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The role of tenascin C under hypoxic and hypertrophic conditions - in vitro H9c2 rat cardiomyoblasts model and potential miRNA targeting approach

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Background: Re-expression of Tenascin C (TN-C) following myocardial infarction and chronic hypertension is associated with the marked increase in myocardial fibrosis and poor cardiac function. However, the underlying mechanism triggers for the expression of TN-C have not been investigated in cardiomyocytes.
Purpose: Therefore, the present study aimed to 1) clarify the effect of hypoxia and hypertrophic agents on 1) TN-C expression and 2) to investigate the interaction of mir-335 expression, as a potential regulator of TN-C in H9c2 cardiomyoblasts.
Methods: H9c2 rat cardiomyoblasts were subjected to two different types of conditions 1) 0-24 hours of glucose and oxygen deprivation (OGD) and 2) incubation with Angiotensin II (Ang II) or Endothelin-1 (ET-1). Furthermore, cardiomyoblasts were cultured with 1 and 10 µg/mL of human TN-C. Cell viability was assessed by annexin V/PI staining and flow cytometry analysis. Total RNA or miRNA was isolated and RT-qPCR was performed to assess the expression levels of Tnc, Mmps, integrins, Erbbs and mir-335 (normalized to Gapdh or snRNA U6, respectively). In addition, TN-C protein was analysed by ELISA in hypoxia treated cells.
Results: Both experimental conditions markedly increased the mRNA expression of Tnc ($p<0.05$, respectively) in H9c2 cardiomyoblast. Cell viability markedly decreased substantially after 16 hours of OGD. In line with this, TN-C mRNA expression was markedly increased following 6 hours OGD, although its protein expression reached highest levels at 16 hours following OGD. Accordingly, prolonged period of hypoxia dysregulated the expression of mir-335, with the first being inversely regulated to TN-C formation. When H9c2 cells were exposed to hypertrophic stressors such as Ang II or ET-1 revealed cellular hypertrophy, associated with a marked increase of Tnc expression compared to control condition ($P<0.05$). Of importance, the administration of human TN-C significantly increased expression of BNP and decreased Mmp2 and Mmp9 ($P<0,05$ vs control, respectively) as well as time and dose dependently modified the expression of integrin α6 and integrin β1.
Conclusions: This is the first study providing evidence that hypoxic and hypertrophic stimuli markedly increase the expression of TNC in cardiomyoblasts. In addition, the expression of mir-335 and TN-C during hypoxia was inversely correlated. Our results provide evidence of TN-C on the deleterious effect in cardiomyoblasts and targeting TN-C by mir-335 might provide a novel therapeutic approach to improve cardiac function following myocardial infarction and chronic hypertension.

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The role of tenascin C under hyperglycaemic and hypertrophic conditions - In vitro H9c2 rat cardiomyoblasts model

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Background: The increase of Tenascin C (TN-C) expression is known to be associated with the maladaptive signalling cascade involving left ventricle remodelling following either myocardial infarction or hypertension. In addition, there is substantial evidence that diabetes induces left ventricle hypertrophy and impaired cardiac function. However, there is a lack of evidence about the impact of hyperglycaemia and cellular hypertrophy stress on the expression of TN-C expression in cardiomyocytes.
Purpose: Therefore, the present study aimed to 1) clarify the effect of hyperglycaemia and hypertrophic agents on 1) TN-C expression and 2) to investigate the effect of TN-C on MMP2, MMP9, Integrin α6 and Integrin β1 in H9c2 cardiomyoblasts.
Methods: H9c2 rat cardiomyoblasts were subjected to two different types of conditions 1) standard control (5.6 mM glucose) and hyperglycaemic (35 mM glucose) and 2) incubation with Angiotensin II (Ang II) and Endothelin-1 (ET-1). Furthermore, cardiomyoblasts were cultured with 1 and 10 ug/mL of human TN-C. The end of the experiments, total RNA was isolated and RT-qPCR was performed to assess the expression levels of Tnc, Mmps and integrins (normalized to Gapdh).
Results: Both experimental conditions markedly increased the mRNA expression of Tnc ($p<0.05$, respectively). Interestingly, hyperglycaemia markedly increased the expression of Tnc ($p<0.05$) compared to controls, while the expression of Mmps and integrins were significantly downregulated ($p<0.05$, respectively). Furthermore, H9c2 cells exposed to hypertrophic stressors such as Ang II or ET-1 revealed cellular hypertrophy, associated with an increase of Tnc expression compared to controls ($P<0.05$). Moreover, human TN-C significantly increased expression of BNP and decreased Mmps ($P<0,05$ vs control, respectively) and reduced the expression of integrins.
Conclusions: This is the first study providing evidence that hyperglycaemia and Ang II markedly increase the expression of TNC. In addition, TNC has a significant regulatory role on the expression of Mmps and integrins. Collectively, these results indicate the fundamental deleterious role of TNC in the progression of cardiac dysfunction in diabetes and hypertension.

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Biophysical consequences of missense mutations associated with Brugada syndrome

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Background/Introduction: Brugada syndrome (BrS) is a rare inherited arrhythmogenic disease, characterized by high risk of sudden death. More than 200 missense mutations in cardiac voltage-gated sodium channel alpha-subunit gene (SCN5A) are described in association with this condition. However, biophysical mechanisms underlying the development of the arrhythmia are identified only for half of the mutations. Furthermore, relations between biophysical features of the mutant channels and clinical phenotype are not completely understood. Here we identified two novel SCN5A mutations in patients with Brugada syndrome. Mutation Y739D in extracellular linker DII_S1-S2 leads to the classical clinical picture of BrS. Mutation A1294G in extracellular linker DIII_S3- S4 is responsible for complex clinical picture including Brugada-like ECG, fibrosis, cardiac dilatation and decreased left ventricular contractility. We explored biophysical properties of the two mutations and estimated their impact to loss-of-function phenotype and pathogenesis of the disease.
Methods: Mutations A1294G and Y739D were introduced in the SCN5A cDNA using site-directed mutagenesis. Sodium currents were recorded at the room temperature in CHO transfected cells. Electrophysiological measurements are presented as mean ±SEM.
Results: Mutation A1294G markedly decreased (62%) the peak current density (-381.8±18.2 pA/pF and 146.1±22.7 pA/pF for WT and A1294G, respectively) and increased two-fold slow time constant of recovery from inactivation. In contrast, mutation Y739D demonstrated a less marked reduction (38%) of INa (-285.6±27.7 pA/pF and -176.9±29.8 pA/pF for WT and Y739D, respectively) and 1.5-fold growth of slow time constant of recovery from inactivation. Patch-clamp measurements did not reveal significant alterations in the kinetic of steady-state activation, steady-state inactivation and the onset of slow inactivation of these mutant channels.
Conclusions: Deceleration of recovery from inactivation and decrease of INa contribute to the loss-of-function phenotype of mutations A1294G and Y739D. A possible relationship between electrophysiological properties and severity of clinical phenotype in patients with SCN5A-related Brugada syndrome is proposed.

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In-silico study of relationship between myocardium activation time and QRS complex width on ECG

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