tight regulation of RANKL expression through the OPG level compensates the inflammatory effect of a RANK-RANKL interaction in EAM.

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Phosphoinositide 3-kinase gamma inhibition as a novel strategy to reactivate targeted autophagy and limit Doxorubicin-induced cardiotoxicity

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Background: Anthracyclines, like doxorubicin (DOX), are among the most potent antitumor drugs. However, their clinical use is hampered by severe cardiotoxicity. We previously demonstrated that inhibition of phosphoinositide 3-kinase γ (PI3Kγ) protects against anthracycline-induced cardiomyopathy (manuscript under revision), but the underlying molecular mechanisms are still unexplored.
Purpose: Here, we further test the hypothesis that anthracycline-damaged mitochondria activate Toll-like receptor 9 (TLR9)/PI3Kγ signaling, which in turn inhibits protective autophagy, thus exacerbating anthracycline cardiotoxicity.
Methods: Neonatal cardiomyocytes (NCMs) were isolated from mice expressing a kinase inactive PI3Kγ (PI3Kγ kinase-dead; KD) and wild-type (WT) controls, and treated with DOX (1 mM) or TLR9 agonist ODN1826 (1 μg/ml) ± PI3Kγ inhibitor AS605240 (500 nM) or TLR9 antagonist ODN2088 (1 μg/ml), for 1 hour before analyzing Akt/mTOR/Ulk-1 signaling. For in vivo studies, WT and KD mice were treated with 4 mg/kg DOX weekly for 3 weeks. Cardiac function was analyzed with echocardiography 6 weeks after the first injection. Electron microscopy (EM) study of morphology and signaling transduction were studied 3 days after the treatment. To investigate the role of protective autophagy in KD hearts, mice were treated with hydroxychloroquine (HCQ) or AAV9-shATG7, which silences the autophagy regulator ATG7 specifically in cardiomyocytes, together with DOX, as described above.
Results: In NCMs, DOX significantly increased the phosphorylation of PI3K downstream targets and autophagy inhibitors, Akt, mTOR and Ulk-1. These effects were completely prevented by the TLR9 antagonist ODN2088, the PI3Kγ selective inhibitor AS605240 and genetic PI3Kg inactivation (KD NCMs). Notably, the TLR9 agonist ODN1826, mimicking mitochondrial DNA (mitoDNA), similarly upregulated Akt/mTOR/Ulk-1 signaling in WT but not in KD NCMs. These results suggest that DOX activates PI3Kγ through mitoDNA/TLR9. In vivo, EM studies showed more abundance of autolysosomes containing injured mitochondria in KD than WT hearts, highlighting the efficient autophagy-dependent disposal of DOX-damaged mitochondria in KD mice. Enhanced activation of PI3Kg-dependent pathway correlated with a significant blunt of autophagy in DOX-treated hearts, whereas inhibition of PI3Kγ promoted autophagy and decreased DOX-induced contractile dysfunction. Finally, inhibition of autophagy by HCQ or AAV9-shATG7 erased the protection in KD mice.
Conclusion: Overall, this study demonstrates that PI3Kγ prevents autophagic disposal of DOX-damaged mitochondria, resulting in cardiomyopathy. We propose PI3Kγ inhibition as a novel strategy to reactivate targeted autophagy and limit cancer therapy-related heart disease.

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Bay 60-2770 attenuates doxorubicin-induced cardiotoxicity by preventing mitochondrial membrane potential loss

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Objectives: The highly effective anti-cancer agent doxorubicin (DOX) induces cardiotoxicity that involves increased oxidative stress, mitochondrial iron overload, DNA damage, necrosis and apoptosis. These effects are also associated with secondary tumorigenicity. Soluble guanylate cyclase (sGC) signaling is protective against cardiovascular disease and can chemosensitize cancer cells. The present study investigated the role of Bay 60-2770, an effective activator of oxidized and heme-free sGC, in alleviating DOX-induced cardiomyopathy (DOX-CM).
Methods: To quantify the protective effect of Bay 60-2770 on DOX-induced cardiac myocyte death and ROS generation, H9c2 cells were treated with 10 μM Bay 60-2770 for 24 h prior to DOX treatment (0.5-10 μM). Mitochondrial ROS and membrane potential were measured with MitoSOX RED and TMRE, respectively. To determine the role of Bay 60-2770 in DOX-CM, Bay 60-2770 was orally administered to 8-week-old male Sprague-Dawley rats 1 hour prior to every DOX treatment. LV dysfunction was then analyzed by echocardiography. The levels of autophagy-related proteins and mitochondrial iron-regulating proteins in the LV were analyzed. The % of autophagosomes in cardiac myocytes was examined by Cyto ID staining.
Results: Bay 60-2770 increased cell viability and reduced DOX-induced oxidative stress in H9c2 cells; these effects were mediated by PKG activation. Mitochondrial ROS and TMRE fluorescence were attenuated by Bay 60-2270 treatment in DOX-treated H9c2 cells. The ratio of Bax/Bcl-2 decreased after pre-treatment with Bay 60-2770, both in vivo and in vitro. Echocardiography demonstrated that pre-treatment with Bay 60-2770 significantly improved LV function. Moreover, Bay 60-2770 enhanced the protein expression of mitochondrial ferritin (MtFt) in heart tissue.
Conclusion: Bay 60-2770 reduces DOX-induced mitochondrial membrane potential loss and subsequent apoptosis. Moreover, improves cardiac function by upregulating MtFt and stimulating autophagy. These novel results highlight the therapeutic potential of Bay 60-2770 to prevent DOX-CM.

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Contribution of oxidized low density lipoproteins to arrhythmogenic cardiomyopathy adipogenesis

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Background: Arrhythmogenic Cardiomyopathy (ACM) is a genetic condition characterized by progressive fibro-fatty replacement of the ventricular myocardium and malignant arrhythmias. We recently showed that Cardiac Mesenchymal Stromal Cells (C-MSC) in ACM hearts differentiate to adipocytes, through the activation of PPARγ.
The variable penetrance and expressivity of ACM suggest the involvement of co-determinants. Physical exercise is the only accepted co-factor. Strong physical activity increases oxidative stress (OxS). 13HODE, a component of oxidized low density lipoproteins (oxLDL), is a marker of exercise-induced OxS, and has been shown in macrophages to produce fat accumulation by increasing the expression of both PPARγ and the oxLDL receptor CD36.
Purpose: To evaluate the effects of OxS and oxLDL on ACM adipogenesis and to dissect underpinning pathways.
Methods: We analyzed plasmas (n=34) and ventricular tissues (n=3) from ACM patients and matched healthy controls (HC). In vitro experiments have been carried out on ACM vs. HC human C-MSC (n=5), while in vivo experiments on the heterozygous PKP2 mouse model (PKP2+/-; n=5).
Results: Significantly higher plasmatic oxLDL were detected in ACM patients compared to HC (290.90±76.31 vs. 122.40±28.73 ng/ml; p=0.04). Moreover, oxLDL levels can discriminate between ACM patients with overt phenotype vs. their asymptomatic relatives carrying the same causative mutations (456.50±187.80 vs. 93.81±33.39 ng/ml; p=0.03). In human ventricular tissue, we observed higher OxS in ACM hearts vs. HC (malondialdehyde positivity 20.26±6.54 fold higher; p=0.004).
In basal conditions, ACM C-MSC also showed higher OxS (diclorofluorescein emission 5.64±0.80 vs. 3.60±0.36 a.u.; p=0.03) and 2.79±1.32 fold higher expression of PPARγ (p=0.04) compared to HC C-MSC. Administration of 13HODE increased lipid accumulation in ACM C-MSC (Oil Red O (ORO) staining 1.28±0.24 fold vs. untreated; p=0.02). On the contrary, treatment with the antioxidant N-Acetylcysteine (NAC) prevented lipid accumulation in ACM C-MSC (ORO staining 0.63±0.16 fold vs. untreated; p=0.03). Lipid accumulation during adipogenic differentiation in ACM C-MSC paralleled with an increased surface expression of CD36 (R2= 0.93; p=0.03).
Despite PKP2+/- mice do not spontaneously accumulate adipocytes in the heart, C-MSC obtained from PKP2+/- mice hearts are more prone to lipid accumulation in vitro than WT cells (ORO staining 99.49±27.36 fold higher; p=0.007). The increase of plasma cholesterol and OxS by administering a high-fat diet, resulted in fibro-fatty substitution in the heart of PKP2+/- mice only (% ORO positive area 0.47±0.15% in PKP2+/- vs. 0.11±0.01% in WT; p=0.009).
Conclusions: Mutations in ACM genes are necessary but not sufficient for ACM complete penetrance. We showed that elevated OxS and oxLDL are important cofactors of adipogenesis. Further investigations could provide new approaches for pharmacological prevention of ACM adipogenic phenotype

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Genetic testing in arrhythmogenic cardiomyopathy: growing complexity embedded in doubts

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Introduction: Arrhythmogenic cardiomyopathy (AC) is a heart muscle disease characterized by life-threatening ventricular arrhythmias and progressive dystrophy of the ventricular myocardium with fibro-fatty replacement. AC is defined as a rare disease due to an estimated prevalence of 1:5000. AC displays mostly an autosomal-dominant transmission and incomplete penetrance. It is considered a disease of desmosomes, however about 1% of AC patients harbor a mutation in non-desmosomal proteins such as CTNNA3, TMEM43, RYR2, TTN, LMNA, DES, PLN, SCN5A. Non-desmomal proteins mutation frequency in AC patients might be underestimated due to gene size-related sequencing and interpretation difficulties.
The aim of this study was to assess the frequency of non desmomal rare variants in AC patients.
Methods: 189 consecutive AC index cases, fulfilling revised 2010 Task Force Criteria, underwent DNA sequencing on the MiSeq platform (Illumina) using the Trusight Cardio panel (174genes). Variant selection was based on current ACMG guidelines: a minor allele frequency (MAF) <0,0001 thresold in the general population, amino acid conservation across species and pathogenicity based on at least two in silico prediction algorithms. Cascade genetic screening in probands' families was performed to study the segregation of rare variants and their impact on the clinical phenotype.
Results: Almost 45% (85/189) of AC index cases carried a rare nucleotide variant in AC-related genes: 40% (76/189) were desmosomal variant carriers and 5% (9/189) displayed rare variants in non-desmosomal AC-related genes (CTNNA3, DES, LMNA, SCN5A, TMEM43). Specifically, 18 were single-variant DSP carriers, 9 DSG2, 23 PKP2, 7 DSC2, 3 JUP, and 16 patients were compound or digenic heterozygous carriers in desmosomal genes.
All 85 genotype positive but also 104 genotype negative AC cases carried rare variants in AC-unrelated genes, mostly encoding sarcomeric proteins (OBSCN, MYH7, MYBPC3) or ion channels subunits (CACNA1B, CACNA1C, SCN10A, KCNQ1, KCND3). Interestingly 26% (51/189) of patients carried at least a rare variant in the TNN; its significance was assessed by cascade genetic screening.
Conclusions: Analysis of a large AC cohort reveals a combination of multiple rare variants in AC related and unrelated genes. Preliminary data demonstrate a high frequency of rare TTN variants in AC patients, which role in the etiopathogenesis of AC as modulatory factor will be clarified by