These research projects, undertaken in partial fulfillment of the requirements for the MD degree at Sackler Faculty of Medicine, Tel Aviv University in 2012-2013, were considered the most outstanding of the graduating class

Mast cell inhibition attenuates myocardial damage, adverse remodeling and dysfunction during fulminant myocarditis in the rat

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Background: Myocarditis is a life-threatening heart disease characterized by myocardial inflammation, necrosis and chronic fibrosis. While mast cell inhibition has been suggested to prevent fibrosis in rat myocarditis, little is known about its effectiveness in attenuating cardiac remodeling and dysfunction in myocarditis.

Objectives: We sought to test the hypothesis that mast cell inhibition will attenuate the inflammatory reaction and associated left ventricular (LV) remodeling and dysfunction after fulminant autoimmune myocarditis.

Methods: To induce experimental autoimmune myocarditis, we immunized 30 rats with porcine cardiac myosin twice at 7 day intervals. On day 8 animals were randomized into treatment either with an intraperitoneal (IP) injection of 25 mg/kg of cromolyn sodium (n=13) or an equivalent volume (~0.5 ml IP) of normal saline (n=11). All animals were scanned by serial echocardiography studies before treatment (baseline echocardiogram) and after 20 days of cromolyn sodium (28 days after immunization). Furthermore, serial cardiac magnetic resonance was performed in a subgroup of 12 animals. After 20 days of treatment (28 days from first immunization), hearts were harvested for histopathological analysis.

Results: Echocardiography showed that cromolyn sodium prevented LV dilatation and attenuated LV dysfunction, compared with controls. Postmortem analysis of hearts showed that cromolyn sodium reduced myocardial fibrosis, as well as the number and size of cardiac mast cells in the inflamed myocardium, compared with controls.

Conclusions: Our study suggests that mast cell inhibition with cromolyn sodium attenuates adverse LV remodeling and dysfunction in myocarditis. This mechanism-based therapy is clinically relevant and could improve the outcome of patients at risk for inflammatory cardiomyopathy and heart failure.

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Effect of mesenchymal stroma cells on cardiac remodeling and function in doxorubicin-induced cardiomyopathy in the rat

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Background: One of the most significant complications of anthracycline therapy in patients with cancer is heart failure (HF) due to cardiotoxicity and dilated cardiomyopathy (DCM).

Objectives: We aimed to test the hypothesis that intravenous (IV) infusion of mesenchymal stem cells (MSCs) would ameliorate cardiac function in a rat model of doxorubicin (DOX)-induced DCM.

Methods: Cardiotoxicity and DCM were induced in 40 female Sprague-Dawley (SD) rats by weekly intraperitoneal (IP) injections of DOX 3 mg/kg for 5 weeks (cumulative dose 15 mg/kg). One week and 2 weeks later, the rats were allocated to receive either two IV infusions of MSCs (1×10^6) or saline.

Results: Due to DOX administration, all animals developed DCM, characterized by a pathological increase in left ventricle (LV) systolic area ($P < 0.002$) and LV mass ($P < 0.0001$). Surprisingly, MSC therapy was associated with deterioration in fractional shortening (-Δ 7.7 ± 4.5% vs. 9.7 ± 5.6%, for controls, $P = 0.02$), fractional area change (-Δ 9.5 ± 3.4% vs. 3.6 ± 3.6%, for controls, $P = 0.01$), and increase in LV systolic area vs. controls (Δ 24.4 ± 9.7% vs. -0.97 ± 6.9%, $P = 0.04$). Furthermore, whereas MSC therapy increased angiogenesis by 44% (11.7 ± 1.4 vs. 8.1 ± 0.9 vessels per mm^2, $P = 0.05$), it reduced macrophage accumulation by 65% (Dox+MSC 7.00 ± 0.86 vs DOX+saline 19.9 ± 2.36 cells per mm^2, $P < 0.001$).

Conclusions: IV MSC therapy accelerated LV dysfunction in anthracycline-induced cardiomyopathy in the rat. These

unexpected findings warrant caution against non-selective MSC therapy for anthracycline-induced DCM.

Klotho's structure-function relationship: Is enzymatic activity essential for growth inhibitory activity in human breast cancer cells?

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Background: Klotho is a transmembranal protein that can be shed and acts as a hormone. Deletion of klotho in mice leads to a phenotype that resembles human aging [1]. Klotho is a co-receptor for FGF23, thus regulating phosphate homeostasis. It also inhibits the insulin-like growth factor (IGF-1) pathway. We were the first to identify klotho as a tumor suppressor. Its levels are reduced in various tumors, including breast and pancreas. Moreover, treating cancer cells with klotho inhibits the IGF-1 pathway and slows cells growth in vitro and in vivo [2-4].

Objectives: As klotho shares sequence homology with the glycoside hydrolase family, it is predicted to possess enzymatic activity [1,5]. We explored whether klotho tumor suppressor activity is associated with its putative enzymatic activity.

Methods: Our computerized structure model of klotho indicated that glutamate at position 416 and aspartate at position 240 are essential for its enzymatic activity. We therefore generated mutated klotho at these positions. The effects of the mutated proteins on breast cancer cell proliferation, IGF-1 and FGF23 pathway activation were examined.

Results: Surprisingly, mutated klotho, which are expected to be enzymatically inactive, inhibited proliferation of breast cancer cells similar to wild-type protein. However, mutated klotho showed reduced ability to inhibit IGF-1 and to serve as a co-receptor for FGF23.

Conclusions: Our findings suggest that the tumor suppressor activity of klotho may be independent of its putative enzymatic activity and is not mediated through inhibition of the IGF-1 pathway. As klotho is a hormone, its administration is potentially feasible and may serve as a novel therapy for breast and other cancers. Mutated klotho may be safer as it is predicted not to affect phosphate homeostasis.

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Erratum

A mistake occurred in the numbering of pages from the April to September issues. The numbers have been adjusted, including all cross-references, and appear correctly online.

Capsule

Usp16 contributes to somatic stem cell defects in Down's syndrome

Down's syndrome results from full or partial trisomy of chromosome 21. However, the consequences of the underlying gene-dosage imbalance on adult tissues remain poorly understood. Adorno et al. show that in Ts65Dn mice, which are trisomic for 132 genes homologous to genes on human chromosome 21, triplication of *Usp16* reduces the self-renewal of hematopoietic stem cells and the expansion of mammary epithelial cells, neural progenitors and fibroblasts. In addition, Usp16 is associated with decreased ubiquitination of Cdkn2a and accelerated senescence in Ts65Dn fibroblasts. Usp16 can remove ubiquitin from histone H2A on lysine 119, a critical mark for the maintenance of multiple somatic tissues. Down-regulation of Usp16, either by mutation of a single normal *Usp16* allele or by short interfering RNAs, largely rescues all of these defects. Furthermore, in human tissues over-expression of USP16 reduces the expansion of normal fibroblasts and postnatal neural progenitors, whereas down-regulation of USP16 partially rescues the proliferation defects of Down's syndrome fibroblasts. Taken together, these results suggest that USP16 has an important role in antagonizing the self-renewal and/or senescence pathways in Down's syndrome and could serve as an attractive target to ameliorate some of the associated pathologies.

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Eitan Israeli

"Lower your voice and strengthen your argument"

Lebanese proverb