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Heart diseases

ROLE OF RESIDENT MACROPHAGES AND FIBROBLASTS ON HEART REMODELING AND HEALING AFTER MYOCARDIAL INFARCTION: THE CONTRIBUTION OF THE GAS6-TAM SYSTEM

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1. Summary

Despite reperfusion and pharmacological therapy, the incidence of left ventricular (LV) remodeling in ST-segment elevation myocardial infarction (STEMI) is high. New therapeutic targets are necessary to prevent heart failure and death. The resident macrophage and fibroblast have a key role in inflammation and tissue repair by interacting with paracrine signals. The Gas6-TAM system of ligands and receptors is involved in tissue repair, regulates the elimination of damaged cells, and modulates fibroblast function, but their role in the healing process after STEMI is unknown. We have studied the modulation of resident macrophages and fibroblasts by the Gas6-TAM system in LV remodeling in human and experimental STEMI.

The role of resident macrophages and the interaction with fibroblasts were experimentally characterized in models of isoproterenol-induced cardiomyopathy and myocardial infarction in mice deficient in cardiac macrophages and controls. The role of the Gas6-TAM system in the differentiation of myofibroblasts was evaluated by combining mutations in the Axl, Mertk and Gas6 genes with mice deficient in resident macrophages, studied with advanced imaging and histology techniques focusing on LV remodeling. At the same time, in a clinical study we measured the levels of Axl, Mertk and Gas6 in serum in a series of patients admitted with first STEMI. The relationship with LV remodeling and interstitial fibrosis in the non-infarcted myocardium was evaluated by cardiac magnetic resonance imaging (CMR), performed at admission and at 6 months.

Our hypothesis was that the Gas6-TAM system would be activated and would modulate the function of resident macrophages and fibroblasts in tissue repair after myocardial infarction, and thus could become a therapeutic target to prevent pathological LV remodeling in STEMI.

1.1. Aims

1. To study the contribution of resident macrophages in LV remodeling after STEMI and in the model of pressure overload induced by isoproterenol.
2. To study the role of the Gas6-AXL axis in the activation of pro-fibrotic myocytes after experimental myocardial infarction and in the isoproterenol model.
3. Determine the effect of inhibition of the AXL pathway in cardiac remodeling and

fibrosis after myocardial infarction.
4. Determine the serum levels of Gas6 and the soluble forms of its TAM receptors in the acute phase and the 6 months following the STEMI, and compare them with the values obtained in healthy controls adjusted for age and classical risk factors.
5. Determine if serum levels of Gas6 and / or Axl and Mertk correlate with adverse LV remodeling or increased interstitial fibrosis using CMR.

1.2. Study design

Experimental studies

We use several strains of mutant mice, Axl KO, Mertk KO, Gas6 KO and CD169-DTR. For CD169-DTR animals, the diphtheria toxin injection was planned on days 2, 5 and 7 days up to 14 days (10 ng/g). This treatment has been shown to suppress CD169 positive cells. The negative DTR controls were injected with the same dose of diphtheria toxin.

Murine model of myocardial infarction.

Two models of ischemia-reperfusion and myocardial infarction were created with temporary (45') and permanent occlusion of the anterior descending artery respectively. Cardiac function was measured by echocardiography before the infarction and at 28 days in the different groups. After the sacrifice of the mice, we performed routine section procedures and histological analysis. The histological sections of the hearts were stained with Masson's trichrome and picrosirius red, and examined twice blindly.

Isoproterenol model.

Isoproterenol was administered by ALZET peristaltic pumps that continuously release isoproterenol in the subcutaneous space of the animal for 14 days. A flow of 0.25 ml/h of a drug solution of 25 mg/kg/day was used. Animals were monitored continuously and sacrificed on day 14. Relevant tissue samples were collected and characterized. Several parameters of TAM signaling activation have been evaluated, including Mertk and Axl phosphotyrosines, AKT phosphorylation and STAT1 and 3 activation. In addition, parameters of a profibrotic phenotype, including TGF-beta, fibronectin, collagen I and III and angiotensin I were determined. These analyzes defined whether the Gas6 / Mertk axis controlled the activation of cardiac myofibroblasts.

Clinical study

We conducted an observational and prospective case-control study. We included 117 patients with a first STEMI treated with primary angioplasty. Serum was obtained 24 hours, 7 days and 6 months after the infarction. Cardiac function, infarct size and interstitial fibrosis of the non-infarcted myocardium were evaluated with cine CMR, T2 and T1 mapping and late gadolinium-enhanced techniques. The levels of the different components of the Gas6-TAM system were measured in the serum by ELISA techniques.

2. Results

2.1. Role of macrophages in cardiac remodeling after myocardial infarction.

The contribution of cardiac macrophages to remodeling after a heart attack was studied using the CD169DTR line that allows macrophage depletion at the desired time. Depletion before the infarction, three or seven days after the infarction showed that the absence of macrophages soon after the infarction caused serious damage to the myocardium and high mortality (Fig. 1-B). That is why we established a system to eliminate macrophages 7 days post-infarction by permanent ligation. With this model we measured cardiac function by ultrasound and remodeling (fibrosis) at 28 days. The results show a clear effect of this late depletion in cardiac remodeling that is reflected in increased fibrosis, and reduction in myocardial contractility (Fig. 1C-D), with decreased ejection and shortening fractions and systolic volume (Fig. 1E).

2.2. Role of TAM receptors in the control of post-infarct cardiac remodeling.

We have specifically studied the Mertk and Axl receptors using mice deficient in these receptors. In the ischemia-reperfusion model, Mertk -/- mice, which do not have this phagocytic receptor in macrophages, there is a significant and marked increase in infarct size at 24 hours post-infarction, which is accompanied by a notable increase in early mortality (3-5 days post-infarction). The permanent coronary occlusion model also showed a marked increase in fibrosis in the heart of the mutant mice (Fig. 1F-H), indicating a clear deficiency in normal healing in the absence of this receptor.

The Axl receptor and its ligand, Gas6, also control phagocytosis and are located in the cells of the immune system. In the acute reperfusion infarction model, both mutants show an increase in the area of myocardial infarction (Figure 2A). This observation

contrasts with a reduction in the area of infarction in conditions of permanent coronary occlusion (Figure 2B), indicating that the inflammatory component in reperfusion conditions can be decisive in early post-ischemic stages in the absence of Axl.

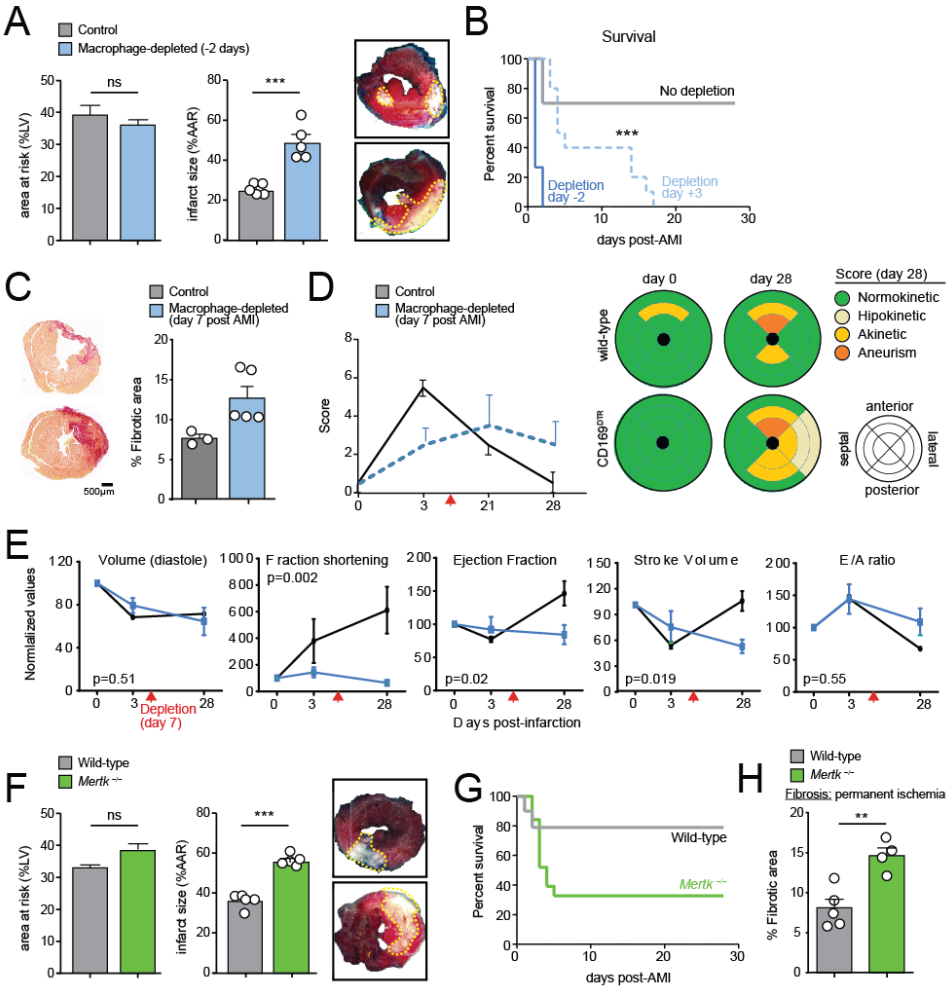

Figure 1

However, the reduction in fibrosis does not prevent further loss of cardiac function (ejection fraction and number of affected VI segments) (Figure 2C). In summary, these data suggest an important cardioprotective role of phagocytic resident macrophages in the acute phase of the infarction.

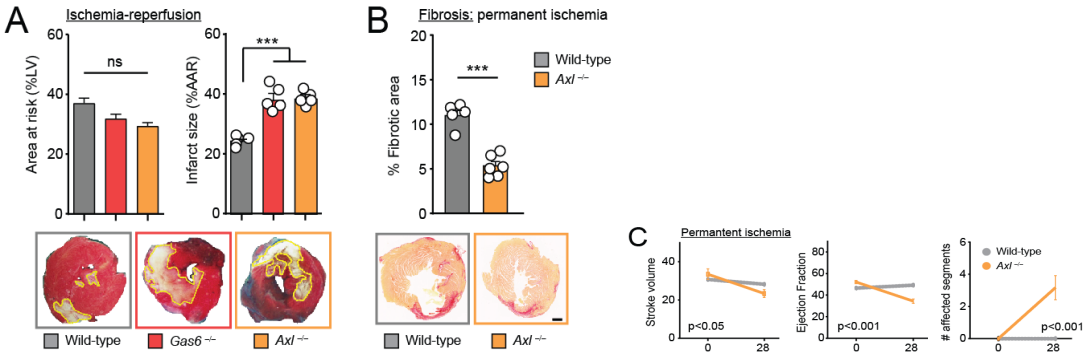

Figure 2

2.3. Effect of isoproterenol in mice deficient in Axl and Mertk

The animals were treated by continuous infusion of isoproterenol for 14 days and then sacrificed. Tissue samples were collected and characterized. The mice suffer from cardiac hypertrophy induced by the drug. An example of each group studied is shown (Figure 3A). The relative weight of the organ is greater in those treated with isoproterenol in the three groups studied (*p <0.05; **p <0.01 with respect to the animals treated with vehicle) (Figure 3B).

In animals KO for Axl, a larger heart size can be seen at baseline, although not significantly. After isoproterenol, KO animals for Axl have a greater relative weight of the heart than in wild animals (&&, p <0.01).

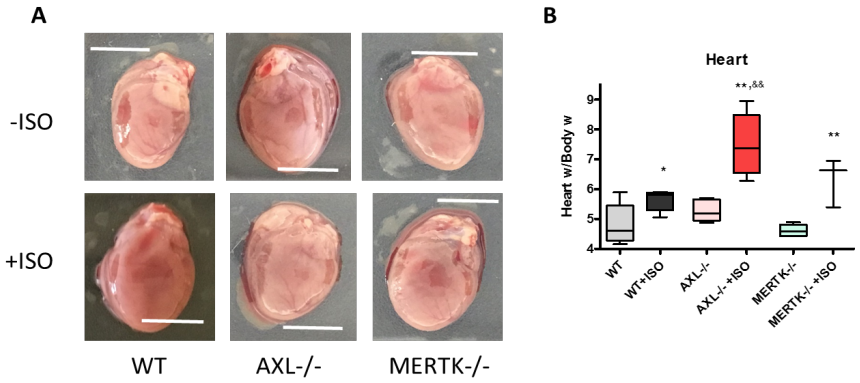

Figure 3

We studied the degree of cardiac interstitial fibrosis after isoproterenol with specific stains for collagen (Figure 4). An example of a histological sample of each group is shown. Mice deficient in Axl and Mertk had a certain degree of fibrosis in the absence

of treatment compared to control mice (& p <0.05; &&& p <0.001). This fibrotic area increased significantly after isoproterenol treatment in control mice and Mertk -/- (*p <0.05 compared to animals of the same genotype treated with vehicle). Surprisingly, a smaller area of fibrosis is seen in KO animals for Axl.

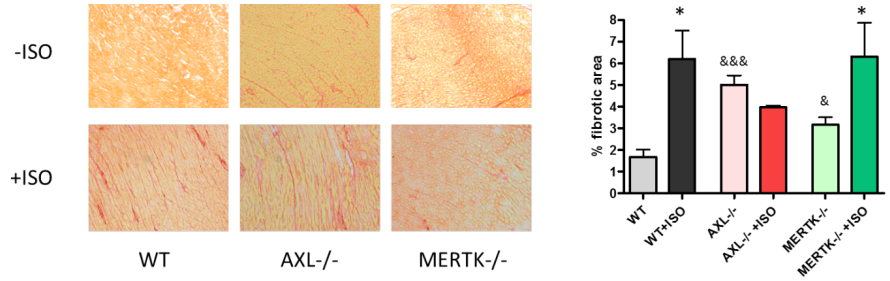

Figure 4

The expression of genes associated with fibrosis in Axl-deficient animals and control animals was determined. While the a-SMA myofibroblast marker levels are maintained in control mice, these levels increase in KO mice for Axl. However, the expression of one of the components of the fibrotic extracellular matrix, collagen A1, is clearly increased in wild-type mice compared to those deficient in Axl. The low expression and lack of induction of the macrophage chemoattractant factor in Axl-deficient animals is remarkable. The significance is indicated by &, p <0.05 with respect to the wild group; * p <0.05 and ** p <0.01 with respect to animals of the same genotype treated with vehicle (Figure 5).

2.4. Results of the clinical study in STEMI

A total of 20 patients presented adverse LV remodeling (Δ iVTDVE ≥20%). In the multivariate analysis that included arterial hypertension, initial EF, baseline creatinine, change in nT1 and segments with microvascular obstruction, only the last two were predictors of adverse remodeling. A progressive increase in Axl values was observed in patients with respect to controls (Figure 6A). In a retrospective cohort (N=227), Axl values were independent predictors of LV remodeling. However, in the prospective cohort (N = 117), patients with adverse remodeling had overall only higher values of Gas6, p <0.05 (Figure 6B), but none of the TAM receptors did correlate with LV volumes or with the infarct size by CMR.

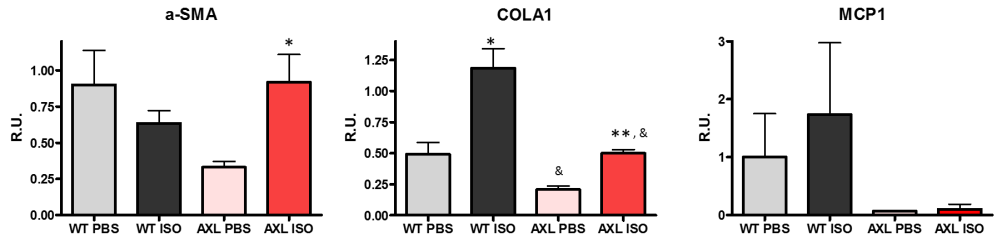

Figure 5

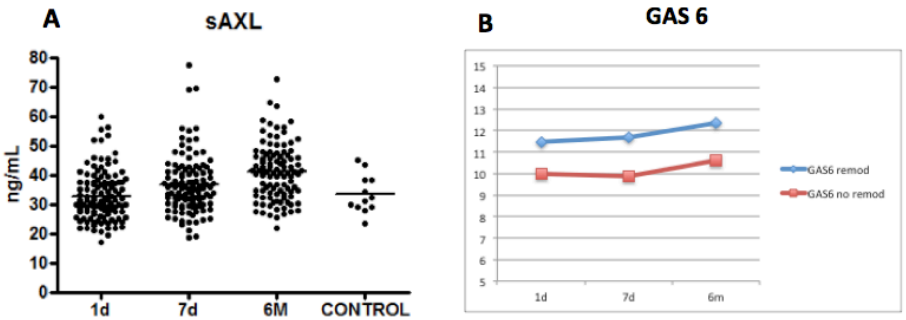

Figure 6

Axl values were correlated with extracellular volume (VEC) in the remote myocardium not infarcted in both the acute phase and at 6 months. In contrast, Mertk values were inversely correlated with VEC at 6 months (Figure 7).

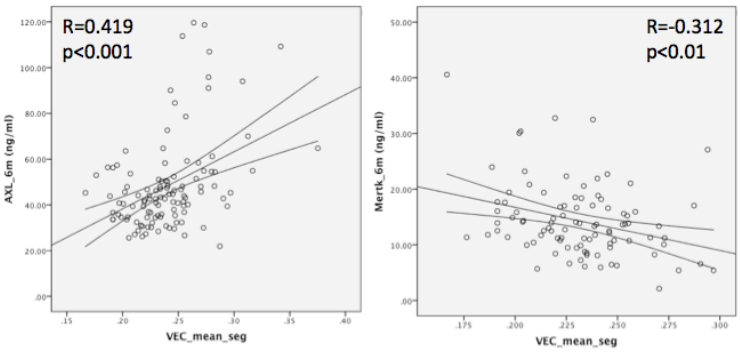

Figure 7

Finally, in both the prospective and the retrospective cohort, higher serum Axl values were observed in patients presenting with heart failure during admission (Killip> 1). In summary, in patients with IAMCEST, serum Axl values appear to have a greater correlation with the pathophysiological processes related to adverse ventricular remodeling, such as heart failure and interstitial myocardial fibrosis.

3. Relevance and possible future implications

In the experimental model of infarction, macrophages and the modulation of their phagocytic Gas6-TAM receptors have an essential role in myocardial repair. The suppression of these receptors has deleterious effects on fibrosis, myocardial remodeling and the recovery of systolic function. In the pressure overload model, Axl deletion is associated with increased hypertrophy but not with increased interstitial fibrosis. However, in humans with STEMI, the serum levels of the components of the Gas6-TAM system did not correlate with the size of the infarction, and only Axl in the retrospective series was associated with adverse LV remodeling. Gas6 and Axl levels correlated with the expansion of the extracellular matrix in the remote myocardium in the acute phase and at 6 months. In addition, in line with the increase in infarct size observed in the Mertk deletion model, in humans an inverse correlation was observed between serum Mertk levels and the increase in the extracellular matrix in the remote non-infarcted myocardium.

Finally, this work shows that the study of the changes observed in the non-infarcted remote myocardium through the characterization of nT1 and VEC with CMR improve the prediction of LV ventricular adverse remodeling, beyond the quantification of the infarct scar. These preliminary findings deserve a more detailed mechanistic study in future research projects, both at the right time after the infarction to modulate the function of the macrophages and the relevance, mainly of Axl and Mertk, in the development of ventricular remodeling.

4. Scientific bibliography generated

Mitochondrial adaptations of the growing heart. In Press. Trends in Endocrinology and Metabolism 2020. Sanchez-Diaz M, Nicolás-Ávila JA, Cordero MD and Hidalgo A.

Programmed 'disarming' of the neutrophil proteome reduces the magnitude of inflammation. Nat. Immunol. 2020. Adrover J, Aroca-Crevillén A, Crainiciuc G, Ostos F, Rojas-Vega Y, Rubio-Ponce A, Cilloniz C, Bonzón-Kulichenko E, Calvo E, Rico D, Moro MA, Weber C, Lizasoaín I, Torres A, Ruiz-Cabello J, Vázquez J, Hidalgo A.

Serum levels of Growth Arrest-Specific 6 (GAS6) Protein and soluble AXL in Patients with ST-segment elevation Myocardial Infarction. European Heart Journal: Acute Cardiovascular Care 2019;8(8):708-716. Caldentey G, García De Frutos P, Garabito M, Berruezo A, Bosch X, San Antonio R, Flores E, Perea RJ, De Caralt TM, Rodríguez J, Ortiz-Pérez JT.

Thrombocytopenia-associated mutations in the Ser/Thr kinase MASTL deregulate global phosphorylation and actin cytoskeleton dynamics in platelets. The Journal of Clinical Investigation (2018) 128:5351-5367. Hurtado, B, Trakala, M, Ximénez-Embún, P, El-Bakkali, A, Sanz-Castillo, B, Álvarez-Fernández, M, Sánchez-Martínez, R, Martínez, L, Muñoz, J, García de Frutos, P, Malumbres, M

CD69 plays a beneficial role in ischemic stroke by dampening endothelial activation. Circulation Research (2019) 124:279-291. Brait, VH, Miró, F, Pérez-de-Puig, I, Notario, L, Hurtado, B, Pedragosa, J, Gallizioli, M, Jiménez-Altayó, F, Arbaizar-Rovirosa, M, Otxoa-de-Amezaga, A, Monteagudo, J, Ferrer-Ferrer, M, de la Rosa, X, Bonfill-Teixidor, E, Salas-Perdomo, A, García de Frutos, P, Lauzurica, P, Planas, AM

A functional role of GAS6/TAM in non-alcoholic steatohepatitis progression implicates AXL as therapeutic target. Cell Mol Gastroenterol Hepatol 2019;9:349-368. Tutusaus, A, de Gregorio, E, Cucarull, B, Cristóbal, H, Aresté, C, Graupera, I, Coll, M, Colell, A, Gausdal, G, Lorens, JB, García de Frutos, P, Morales, A, Marí, M

A network of macrophages supports mitochondrial homeostasis in the heart. Under review, Cell 2019. Nicolás-Ávila JA, Lechuga-Vieco, Díaz E, Santiago DJ, Través PG, Rubio A, García-Marqués F, A-González N, Li JL, Quintana JA, González-Guerra A, Crainiciuc G, Guzmán G, Larrazabal J, Herrero E, Alegre-Cebollada J, Lemke G, Borreguero JJ, Reyes G, Castrillo A, Vázquez J, Priori S, Bernal JA, Enríquez JA and Hidalgo A.

Risk stratification in patients discharged for STEMI. Ejection fraction by echocardiography as the gatekeeper for a selective use of cardiac magnetic resonance. Journal of the American College of Cardiology Cardiovascular Imaging. Under review. Marcos-Garces V, Gavara J, Lopez-Lereu MP, Monmeneu JV, Rios-Navarro C, De Dios E, Pérez N, Merenciano H, Gabaldón A, Cànoves J, Racugno P, Gonzalez J, Bonanad C,

Minana G, Nuñez J, Moratal D, Chorro FJ, Valente F, Lorenzatti D, Rodríguez-Palomares JF, Ortiz-Pérez JT, Bodí V.