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Article

Krüppel-like Factor´s Expression Profile and Gene Interactions following Isoproterenol-Induced Myocardial Damage

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Abstract: Myocardial damage is a precursor to pathological cardiac hypertrophy, a process known to involve members of the Krüppel-Like Factors (KLFs) family, which possess pro-hypertrophic and anti-hypertrophic roles. Our study delved into the molecular mechanisms underlying KLFs-cardiac hypertrophy interplay following myocardial infarction. We induced myocardial damage in rats using isoproterenol. Total RNA was extracted from the left ventricle and Quantitative Real Time RT-PCR was conducted to assess the expression of KLFs, cardiac commitment genes, inflammatory markers, and certain conduction-related genes. We developed a computational approach to construct a KLFs interacting gene network. Initial results revealed early expression (2-3 days after induction) of *Klf3*, *Klf4* and *Klf6*, followed by the expression of *Klf11* and *Klf15* (5-8 days after induction). Finally, *Klf12* and *Klf13* regulators were found upregulated around day 13 after myocardial damage induction. IL-6 was found to be gradually upregulated. Interaction analysis revealed *Klf3*, *Klf8* and *Klf12* interacted with cardiac conduction genes. RT-PCR confirmed up-regulation in cardiac genes linked to electrical function and scar maturation. Our findings underscore the central role of KLFs during the modulation of the cardiac hypertrophic response. Dysregulation of KLF expression resulted in damaged myocardium, participating in the progression of abnormal hypertrophy, highlighting their potential as therapeutic targets for heart diseases.

Keywords: Krüppel-like factors; myocardial damage; cardiovascular diseases; hypertrophy

1. Introduction

Cardiovascular diseases continue to hold the unenviable position as the leading cause of death worldwide(WHO, 2020) Within this group of diseases, prominent exhibitors include ischemic heart disease, stroke, and heart failure, all of which contribute significantly to the global burden of morbidity and mortality(Gaziano et al., n.d.). Among them, ischemic heart disease stands out as one of the most severe because of its rapid onset, particularly when presenting as acute myocardial infarction, claiming over a million lives each year in the United States alone (Mechanic et al., 2022). The sudden interruption of blood flow to the cardiac muscle triggers hypoxia, and if blood supply is not promptly restored, it culminates in tissue necrosis with irreversible loss of cardiomyocytes. From this moment on, there is a series of pathophysiological response, from which the myocardial tissue

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undergoes structural remodeling (pathological hypertrophy) to maintain cardiac contraction and prevent progression towards heart failure. This process involves the release of proinflammatory mediators, an increase in the secretion of extracellular matrix, and compensatory hypertrophy of the remaining cardiomyocytes (Ferrini et al., 2019).

The Krüppel-like factors (KLFs) are a family of transcription factors known to be associated with many biological processes, including the regulation of cardiac hypertrophy. These belong to a group of DNA-binding proteins that are part of the zinc-fingers transcription factors family. Each one of them is composed of three zinc fingers of the Cys2His2 type, which are capable of binding to three pairs of bases, respectively, in GC-rich regions, such as the consensus sequences CACCC-, GC-, and GT-box (Oishi and Manabe, 2018). Since their discovery in 1993 up to the current date, a total of 18 KLFs have been reported, each with unique expression patterns and the ability to regulate gene transcription, either activating or repressing, numerous targets involved in physiological and pathological processes at the subcellular level (McConnell and Yang, 2010). These processes encompass growth, differentiation, and cellular apoptosis, as well as the maintenance of specialized tissues (Tetreault et al., 2013).

Moreover, KLFs are key players in the regulation of cardiac hypertrophy, and their activity can have both pro-hypertrophic and anti-hypertrophic effects, depending on the context and interactions with other regulatory factors. Several aspects, including changes in gene expression, modifications to cellular signaling pathways, and alterations to cellular processes within cardiomyocytes, can influence the development of cardiac hypertrophy. For instance, *Klf2*, which is highly expressed in endothelial cells, has been identified as a negative regulator of cardiac hypertrophy (Boon et al., 2007; Li et al., 2021). Previous research has shown *Klf2´*s ability to inhibit TGFβ signaling in endothelial cells by promoting the expression of Smad7 and blocking the activator protein 1 (AP-1) activity [9]. Moreover, *Klf2* induction by Simvastatin, achieved a significant reduction in the expression of hypertrophic genes, such as atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP), and α-myosin heavy chain (α-MHC), in a Transverse Aortic Constriction (TAC) model (Li et al., 2021). In contrast, *Klf5* has been found to be increased in models of cardiac damage, namely end-stage heart failure or diabetic cardiomyopathy(Hoffman et al., 2021; Kyriazis et al., 2021). Additionally, its role as a mediator of hypertrophy has been demonstrated in heterozygous *Klf5* mice, in which a reduced response to Angiotensin II infusion was observed, resulting in less cardiomyocyte growth and fibrosis development, emphasizing the importance of its presence for the onset of hypertrophy (Shindo et al., 2002). The significance of *Klf5* in hypertrophy development lies in its capacity to transactivate targets such as Platelet-Derived Growth Factor (PDGF)-A/B, Egr-1, Plasminogen Activator Inhibitor-1 (PAI-1), inducible Nitric Oxide Synthase (iNOS), and Vascular Endothelial Growth Fac-tor (VEGF) receptors, amongst others, all of which play a pivotal role during cardiovascular remodeling (NAGAI et al., 2005)

In this study, we aimed to investigate the gene expression of specific KLFs following myocardial infarction induction and the development of hypertrophy. Our research group's primary goal is to contribute to the elucidation of the intricate mechanisms underlying KLFs gene regulation during cardiac hypertrophy, as well as their potential links to inflammatory genes and cardiac conduction. By examining the gene expression patterns of KLFs and their interactions with key mediators, we aim to shed light on the regulatory processes governing cardiac hypertrophy.

2. Materials and Methods

2.1. Animal Study Approval

All experiments were carried out using 150g ± 20g female Wistar rats. These were housed in polymethylmethacrylate boxes and kept at 20 – 24 °C in a room with 12-hour light/dark cycle. Food and water were available ad libitum in the cage. Research with animals carried out for this study was performed according to approved protocols (BI21-00006) and animal welfare regulations of Nuevo León Autonomous University´s Institutional Bioethics Committee.

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2.2. Rat model and cardiotomy

For myocardial damage induction, 65 mg/kg of the beta-adrenergic drug, isoproterenol hydrochloride (SKU: I5627 Sigma-Aldrich, St. Louis, MO, USA) was administered intraperitoneally, dissolved in 0.5 mL of physiological solution (0.9% Sodium Chloride) at room temperature, in a single dose. The control group was administered with 0.5 mL of physiological solution intraperitoneally in a single dose. The experimental animals were euthanized under anesthesia by cervical dislocation. To collect the hearts, the thorax was accessed through an inverted T incision and longitudinal sternotomy. The heart was freed by clamping and cutting the great vessels of the corona cordis, allowing the removal of the organ from the rat's body. Myocardial damage was confirmed by H&E staining. This procedure was performed at different time lapses after chemical induction of cardiac insult with isoproterenol.

2.3. Cardiac morphometry and heart volume calculation

Heart´s length, width and height were measured with a digital vernier. Using the ellipsoid volume formula ($V=3/4\pi\times D1\times D2\times L$), heart volumes were calculated and normalized to body weight of each experimental animal.

2.3. RNA Isolation and Quantitative Real-Time RT-PCR

Total RNA from the left ventricle area was extracted with TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc., Waltham, MA, USA), according to the manufacturer's protocol. Complementary DNA (cDNA) was synthesized by reverse transcription reaction with 250 µg of total RNA using SuperScript™ VILO™ cDNA Synthesis Kit (Invitrogen; Thermo Fisher Scientific, Inc.). Real time PCR reactions were amplified and analyzed in triplicate using an Applied Biosystems 7500 Fast Real time PCR thermal cycler and following the SYBR-Green® FAST protocol (Thermo Fisher Scientific, Inc.) under the next reaction conditions: Step 1: 95°C for 20 seconds, Step 2: 40 cycles of 95°C for 3 seconds, Step 3: 40 cycles of 60°C for 30 seconds. Glyceraldehyde-3-phosphate de-hydrogenase (GAPDH) levels were used to normalize the expression of target genes. The relative expression levels of the genes were calculated using the $2^{-\Delta\Delta Ct}$ method. The list of primers sequences for target genes can be found in supplementary material.

2.4. Statistical analysis

The collected data was analyzed using SPSS software version 17.0 (SPSS, Inc. Chicago, IL, USA), data are expressed as mean ± SEM. Statistical significance was determined using one-way ANOVA with Dunnett correction. A P value <0.05 was considered statistically significant. Graphs and visual aids were made using GraphPad Prism Version 5 and the Biorender app, respectively.

2.5. STRING

STRING integrates information from multiple sources, including high-quality experiments, curated databases, and computational predictions, to construct large-scale protein interaction networks. We utilized STRING (version 11.5) with the multiple protein search option to explore their interactions. The resulting interactome was downloaded in TSV format and visualized using Excel. Our investigation primarily focused on the direct interactions involving KLFs; priority was given to those with the highest correlation or weight, and we compared them with relevant literature. To facilitate a more intuitive comparison, we constructed a Venn diagram that depicted the distinct interactions of our genes of interest with diverse KLFs.

3. Results

Female Wistar rats of 150g ± 20g were initially injected with a single interperitoneally dose of isoproterenol (65mg/kg) to induce myocardial damage(Grimm, 1998; Hosseini et al., 2022; Liu et al., 2013). At 24-hour intervals, animals were sedated, euthanized, and subjected to cardiac removal.

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Figure 1A shows the representative surgical removal process. Cardiac hypertrophy was expected to occur in a period of approximately 21 days (Ferrini et al., 2019). Figure 1B shows the comparative of the control heart size, versus heart size at 21 days post-induction.

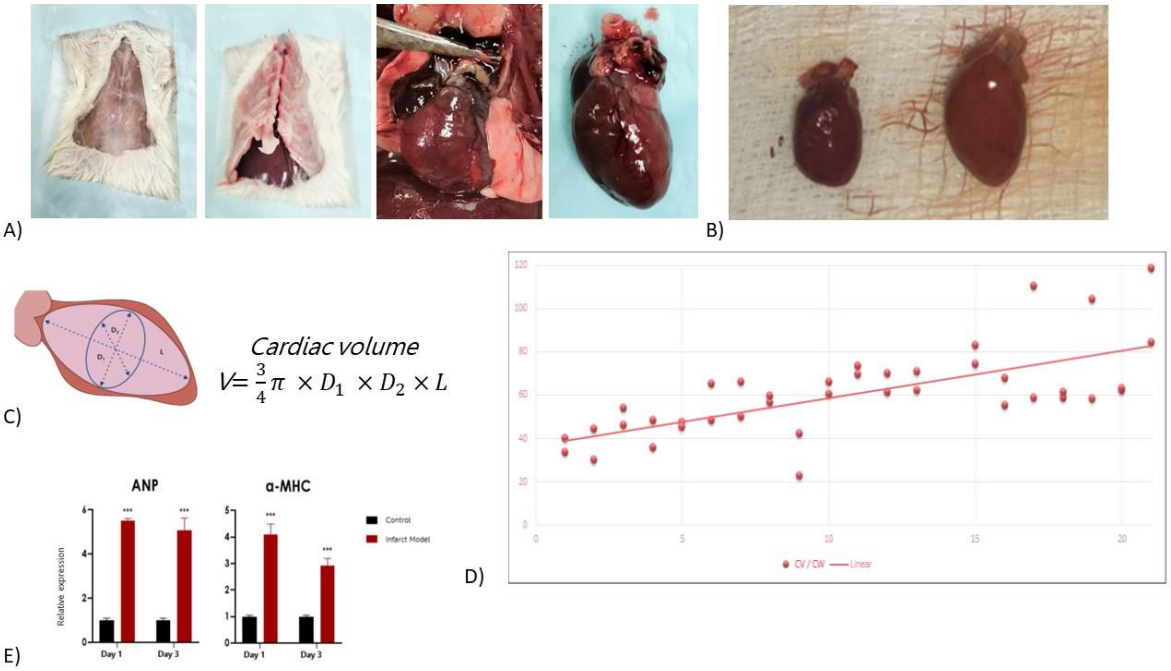

Figure 1. Isoproterenol induced cardiac damage. A) Cardiac surgical removal procedure, B) Left, control vs right, 21-day Isoproterenol induced damage (hypertrophy). C) Cardiac volume schematic and volume formula, D) Time lapse vs. CV/BW plot, E) NPPA and α-MHC expression control vs isoproterenol induced damage.

Before surgery, animals were weighted, and after surgical removal of the organ, the heart was measured for length and width, as well as weighted (weights and measurements can be found in Supplemental Table S1). With the measured variables, cardiac volume was calculated and normalized to the weight of each experimental animal (CV/BW). (Figure 1C) and a graph of the time lapse versus CV/BW was developed (Figure 1D), showing a continuous increase overtime. As a final control of isoproterenol-induced cardiac damage, both ANP and α-MHC, known markers of stress and cardiac hypertrophy, respectively, were shown to increase in expression over time, as seen in Figure 1E.

Since our main goal centered on better understanding the regulation of the Krüppel-like family of transcription factors, and their post-infarction role, we sought to measure their expression levels in combination to the cardiac commitment triad of *Gata4*, *Mef2c*, and *Tbx5* (GMT), which have been shown to reactivate under pathological conditions in the adult heart. As an insult to the heart promotes a state of inflammation, we further measured levels of known inflammatory cytokines *Il-1*, *Il-6*, *Tnf-α*, *Nf-κb*, amongst others.

Figure 2A shows a complete overview (RT-PCR heat map) of the entire KLFs network of transcription factors, in addition to GMT and the inflammatory cytokines. Post-infarction cardiac hypertrophy is segmented into three primary phases: an early inflammatory phase consisting of the first 4 days, followed by a proliferative stage (day 5 – 14) and a final onset of scar tissue maturation up to three weeks from the initial state (Venugopal et al., 2022). Regarding this categorization, we conducted daily assessments of RNA expression from the initial 24 hours following the infarction event and extending up to day seven, which corresponds to the mid-inflammatory phase. Subsequently, samples were evaluated every alternate day for the duration of three weeks, encompassing the entire observation period.

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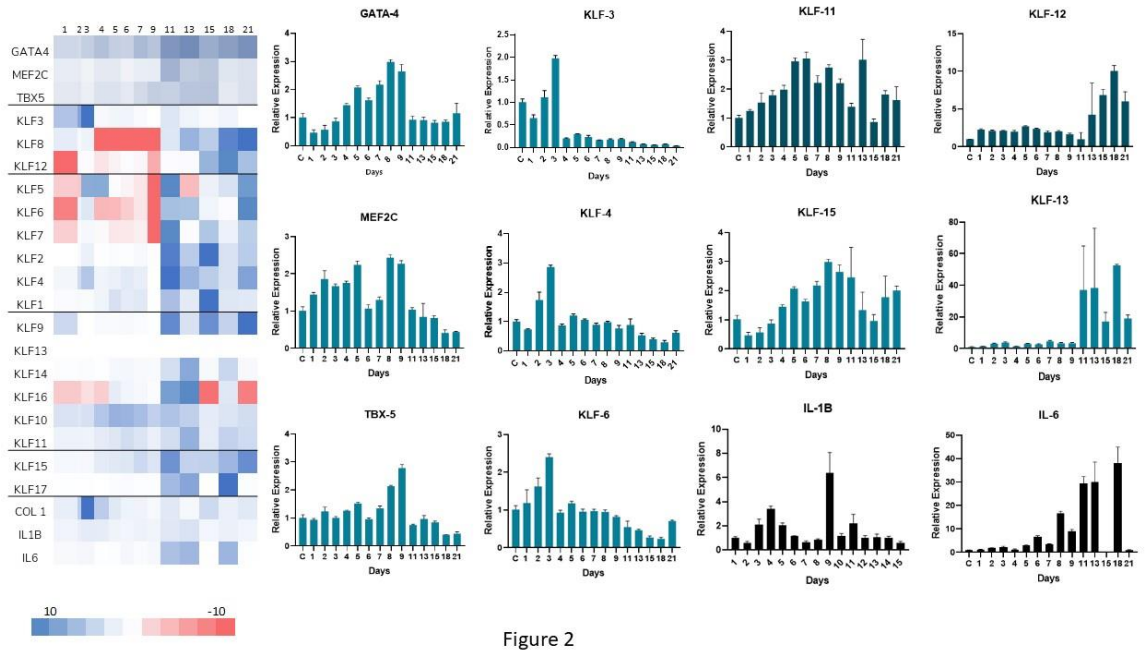

Figure 2

Figure 2. Gene expression profiles. A) Heatmap of representative gene expression for the entire Krüppel-like family and related genes, B) qRT-PCR showed gene expression for KLFs, inflammation and GMT representative of distinctive early-, mid- and late-stage remodeling phases.

Regulatory effects were deemed close to the projected hypertrophic stages (Figure 2B). Several known KLFs related to cardiac function and growth exhibited significant alterations. First, our results showed an early up-regulation of *Klf4*, particularly at days 2 and 3, similarly *Klf3* and *Klf6* also have their highest peaks around day 3. These members of the KLFs are noticeable as they are involved in pro-fibrotic and antiapoptotic signaling. Which could help explain the initial growth effects and fibrosis of the heart. Moreover, through the hypertrophic evolution related to the damage, in the proliferative stage, there is a remarkable upregulation of both *Klf11* at days 5 and 6, and *Klf15* at day 8. It is important to state that *Klf11* is related to TGF-β signaling which can lead to SMAD related cell growth (Pardali et al., 2017; Zhang et al., 2013). Meanwhile, *Klf15* is known directly as a hypertrophy modulator, known to inhibit both *Gata4* and *Mef2c* (Fisch et al., 2007). Consistent with this observation we see a drastic drop in all 3 components of GMT: *Gata4* on day 8 and both *Mef2c* and *Tbx5* on day 9. In the final mature stage, there is an up regulatory effect in *Klf12* and *Klf13*, both pro-apoptotic regulators, which could indicate final patterning. Additionally, a quick look at *Il6* showed a continuous increase in inflammation through the entire remodeling process (Figure 3 and Table 1).

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Figure 3

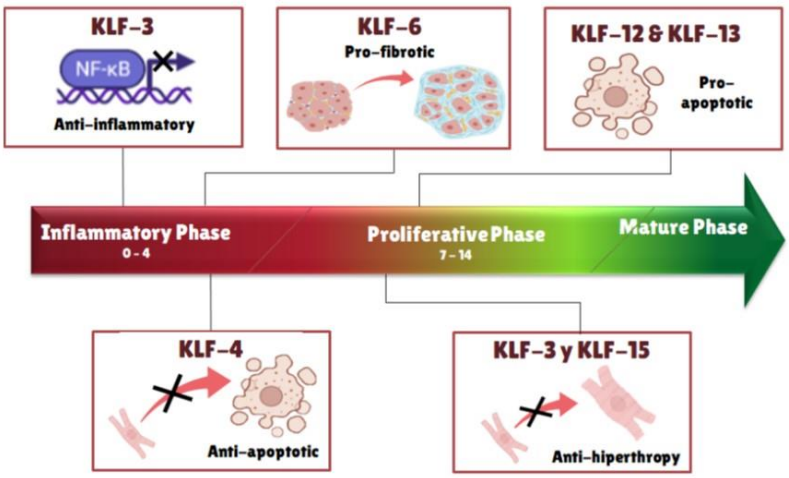

Figure 3. Schematic representation of activation of particular Krüppel-like factors at different stages of remodeling post isoproterenol induced cardiac damage.

Table 1. Krüppel-like factor activation and function related to characteristic members and their potential effects.

Gene	Regulation over time	Max Expression Day	Relative Expression	Potential Effect
KLF-3	Downregulated	3	1.98x	Hypertrophy repressor
KLF-4	Downregulated	2 3	1.7x 2.8x	Antiapoptotic
KLF-6	Upregulated	3	2.4	Profibrotic
KLF-11	Upregulated	5 6 13	2.96x 3.05x 3x	Unknown
KLF-12	Upregulated	16 18	7.2x 9.7x	Proapoptotic
KLF-13	Upregulated	18	53x	Proapoptotic / Anti-inflammatory
KLF-15	Upregulated	8	3x	Hypertrophy modulation

Cardiac function is critically related to its electrical functions. As a first bioinformatic approach, we sought to identify genes interacting with KLFs. Using the STRING database, we constructed rudimentary networks, aimed to initiate the identification of direct correlations between electrical genes. Our initial screening yielded the presence of components related to cardiac electrical function (Na/K pumps, calcium exchangers), Wnt, BMP, SMAD signaling elements, and inflammatory mediators (*Irx3, Acrv2a, Cntn2, Dsg1, Cx40, Cx43, Hcn, Kcna2, Pcp4, Atp2a2, Scl8a1, Scn5a, Tnni3k, Apc, Ryr2, Cacna1g, Bmp2, Bmp4, Gsk3B, Inhba, NfkB1, Ripk1, Smad2, Smad3, Tnfrsf1,Tnf-a*) Figure 4. Next, we proceeded to cluster these results based on the interacting KLFs subtypes. A detailed protein-protein interaction table is presented in Supplementary Table S3. Briefly, each row in the table represents an interaction between two genes, specifically, reciprocal interactions between gene pairs, with the first column indicating protein 1 and the second column indicating protein 2. These interactions were determined through experimental methods or predicted based on various lines of evidence, including chromosomal proximity, gene fusion, phylogenetic co-occurrence, homology, co-expression, experimentally reported interactions, database annotations, and text-mining. Subsequent

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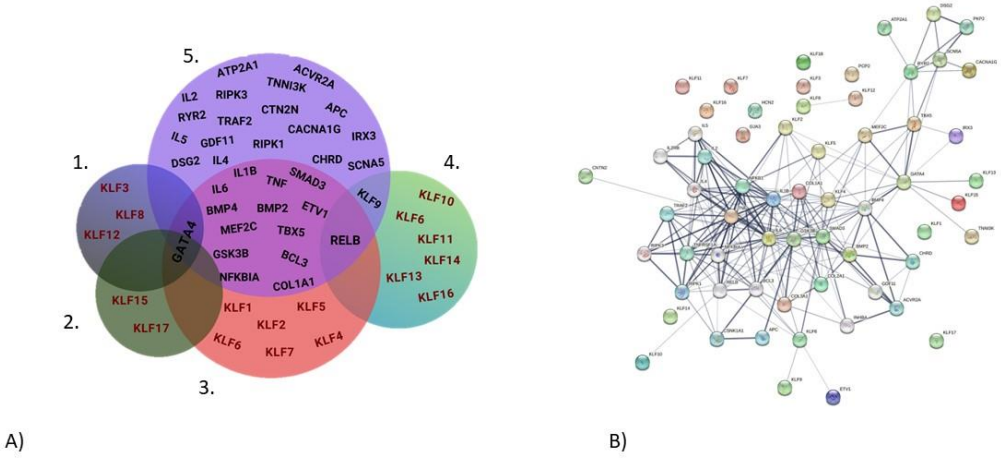
columns contain scores assigned to each type of interaction evidence, and the last column provides a combined score. This table serves as a resource for exploring and prioritizing interactions between genes of interest, leveraging the integration of multiple data sources performed by the STRING database.

Figure 4



A) B)

Figure 4. Left, Venn diagram of interactions given by the predicting string interactome. Right, interactome shows direct and indirect relation of KLF´s, GMT, electrical, and inflammatory genes.

Finally, Figure 5 presents an overview (RT-PCR heat map) of the expression profiles of key components of either cardiac electrical function, WNT, BMP, SMAD signaling and inflammatory mediators; these components as seen in Figure 4 have a relation to the different KLFs. Taking a more particular guise, Figure 4 (right) shows interesting regulatory effects which in part may be driven by the changes in expression of diverse KLFs. Earlier mention *Klf11*, was shown to have its highest expression just around day 9 and was said to have potential regulatory effects over SMAD signaling, through inflammation(Boon et al., 2007; Pardali et al., 2017). In this respect, *Smad2* expression profile shows an important elevation of its expression early on, around day 6, but is quickly suppressed by day 9. Nonetheless, levels of *Smad2* re-activate similarly to those of *Smad3*. Inflammatory cytokines such *Il-6* were shown to elevate at late stages of hypertrophic remodeling, coinciding with elevation of *Klf13* and *Klf12*. Moreover, *Bmp2* expression further correlates with *TGF-b* signaling, in direct relation to SMAD expression(Varga et al., 2021; Zou et al., 2021).

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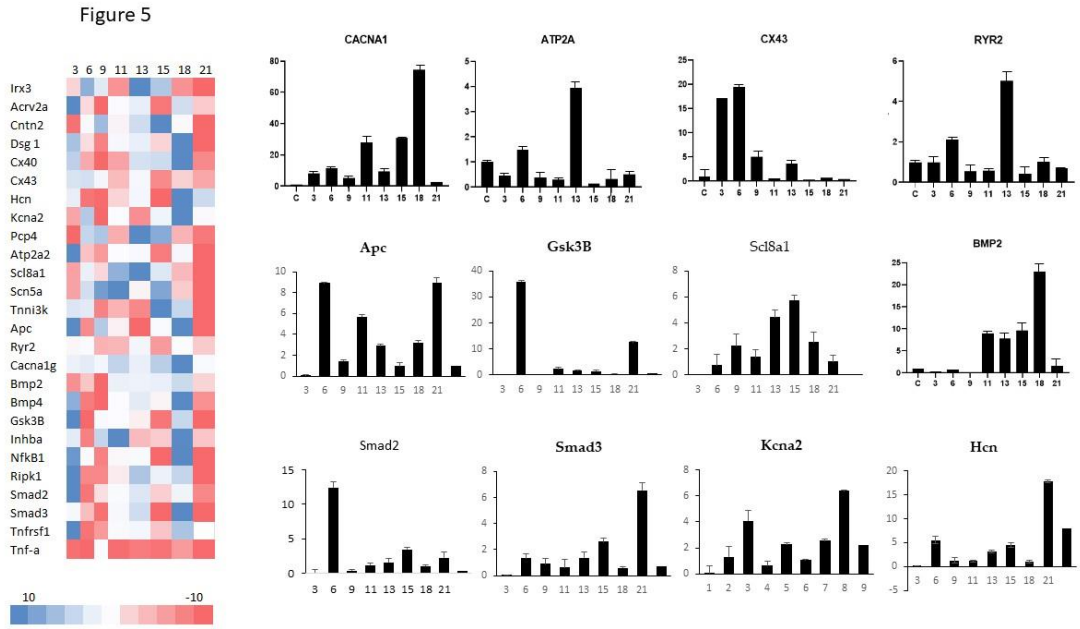

Figure 5. Electrical gene profile, Left-top, shows the representative heatmap expression. Additionally, qRT-PCR graphs plots presenting gene expression profiles.

GATA4 is an important component of the cardiac commitment triad GMT(Christoforou et al., 2013). Its expression was shown at its highest just over a week post myocardial damage, as potential cardiac recovery from damage should take place. Expression of cardiac specific genes such as ion channels *Hcn1* and *Kcna2* showed their highest elevation around day 18. Surprisingly, the sodium calcium exchanger *Scl8a1* was highly expressed earlier, around day 15 before steadily declining its expression.

4. Discussion

The heart is a critical organ in charge of distributing oxygen and nutrients to the entire body. Regrettably, this essential organ lacks the potential to self-regenerate, hence the heart is susceptible to the harsh effects of wear and tear, as well as environmental and genetic degeneration, which in turn leads to exacerbation of an inflammatory state, typically followed by loss of cardiomyocytes, fibrosis, and pathological remodeling (Goradel et al., 2018; Islas and Moreno-Cuevas, 2018; Santoyo-Suarez et al., 2023; Sarre-Álvarez and Cabrera-Jardines, R Rodríguez-Weber, 2018). Given its lack of natural capacity for renewal, it is critical to enhance our comprehension of the progression of these altered deteriorating stages. According to the American Heart Association, myocardial infarction, better known as a heart attack, kills just over 800,000 people in the U.S. alone every year and is considered the leading killer of CVD´s (Tsao et al., 2023). The Krüppel-like factors family has recently received extensive attention, as novel research has centered in their involvement in many processes including embryogenesis and development of diseases related to several organs(Choi et al., 2018; Dabravolski et al., 2022; Leenders et al., 2012; Pabona et al., 2010; Santoyo-Suarez et al., 2023; Shankman et al., 2015; Vinjamur et al., 2014; Yang et al., 2018; Zheng et al., 2009). Isoproterenol is a β-adrenergic stimulant known to induce infarction-like lesions in the myocardium, leading to loss of cardiomyocytes, fibrosis, and remodeling, potentially leading to heart failure (Grimm, 1998). Several studies have confirmed that β-adrenergic stimulation increases cardiac contractility developing maladaptive hypertrophy, led by several mechanisms including enhanced protein synthesis, proto-oncogene expression, elevated oxidative stress, and stimulation of mitogen activated protein kinases (Chowdhury et al., 2013). As earlier stated, post-infarction pathological remodeling typically takes 3 weeks (approximately 21 days). We tested a single intraperitoneal dose of isoproterenol (65 mg/kg), and over the course of 3 weeks, CV/BW more than doubled (Figure 1D). The cardiac hypertrophy

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was further confirmed by the increase in fibrosis, as observed by H&E staining (supplemental Figure 1). The observed pathological maladaptive hypertrophy, as seen in our animal models, is consistent with the earlier studies, wherein high dosages of isoproterenol induced significant irregularities in cardiac architecture, fibrotic infiltration, widening of the interstitium, and loss of cardiomyocytes (Grimm, 1998). Finally, Atrial Natriuretic Peptide (NPPA) is considered a marker for cardiac stress. In clinical settings, NPPA elevation has long been considered a staple of heart failure (Ilatovskaya et al., 2022), as it has been shown to elevate during high demanding workflows, stimulating vasodilation and alleviates hypertension (NISHIKIMI et al., 2006). During a stress-related event such as the one promoted by isoproterenol, myocyte damage induces remodeling and an expected elevation in NPPA (Ilatovskaya et al., 2022), as seen in our results, Figure 1E shows a 5-fold surge in NPPA levels and an initial 4-fold increase in α-MHC expression, both indicative of cardiac damage and heart hypertrophy, respectively.

As we followed myocardial damage over time, GMT alterations were expected. Post infarction-like stimulus is known to induce a strong MAPK kinase signaling cascade. As a result, considerable GATA4 phosphorylation occurs, favoring pro-hypertrophic gene transcription [39]. *GATA4* is a transcription factor that directly regulates the expression of several heart-specific genes, including the α-MHC, troponin C (Tnnc1), troponin I (Tnni3), NPPA, BNP, and ion transport genes, such as the cardiac sodium-calcium exchanger Slc8a1 (Figure 5). Previously, murine research has shown that specific *Gata4* overexpression can cause cardiac hypertrophy, as demonstrated by Liang et al., (2001) who used transgenic animals capable of expressing *Gata4* 2.5 times more than wild type. In Figure 2 we observed this similar increase by day 8. Moreover, *Mef2c* and *Tbx5*, both essential players in *Gata4* regulation show similar trends of upregulation by day 9. Our interactome data (Figure 4B and Supplemental Table S3) further confirmed this interaction and showed a strong *Gata4* relation to *Klf2, 4*, 5, 13 and 15. Moreover, *Klf6* showed involvement with Gata4 *and SMAD3* which are related to SMAD signaling, in addition *Klf6* was further related to *Il-6,* and *Nf-κb (Nf-κb1a,* and *Relb),* all related to inflammatory processes(Zhang et al., 2014). TGFβ, a driver of SMAD signaling, functions as an effector of tissue fibrosis, which can eventually lead to scaring and elevation of collagen related genes (Hu et al., 2018). Both *Klf4* and *6* exhibited early post myocardial damage elevations, potentially leading the way for hypertrophy. Under this perspective, hypertrophy could lead to high levels of fibrosis in the heart, reducing its function and increasing its size. Our data further confirms the activation of *Col1A1* and *Col3A1* (Supplemental Table S3), as expression data show both the elevation of *Klf4* and *6,* as well as elevation of *Bmp2, Il-6,* and *Smad3* (Graphs of Figures 2 and 5). In opposition, around 1 week post myocardial damage, upregulation of *Klf15* peaks, potentially blocking hypertrophy progression(Leenders et al., 2012, 2010; Wenying et al., 2021). *Klf15* has been shown to block *Mef2c* and *Gata4* specific DNA-binding sites, preventing the binding of its coactivator, Myocardin, which works as a hypertrophy antagonist (Prosdocimo et al., 2015). Hence, marking the start of the maturation phase, wherein we can observe an elevation of *Klf12* and *13*. While information regarding KLF12 is limited in its involvement in the downregulation of Survivin, -an antiapoptotic factor- has been demonstrated (Mak et al., 2017). In addition, KLF13, a pro-apoptotic factor, seems to favor homeostatis, as it is potentially inhibiting BCL-XL and consequently triggering cell death. Left Anterior Descending Coronary Artery Ligation (LAD) murine models have shown an increase in *Klf13* expression related to early phase cardiomyocyte death, a contrasting effect to that of *Klf4* (Bayoumi et al., 2018; Zhou and Herring, 2005).

GMT genes contribute to cardiac maintenance, contraction, and growth of the heart (Christoforou et al., 2013; Dirkx et al., 2013; Liu, 2017; Liu et al., 2016). Unsurprisingly, electrical function is also related to these genes. Tbx5 has been related to SRF and CX43 (early activation post myocardial damage), which are directly related to myocardial stress, which, when exacerbated, leads to pathological hypertrophy (Christoforou et al., 2013; Liang et al., 2001). Cardiac muscle contraction (RYR, ATP2A, αMHC) and electrical activity are intrinsically related. Interestingly our findings reveal a sharp decrease in several of these genes, particularly by day 10 (Figure 5), strongly suggesting a decline in function, along with the preceding drop of CX43 expression (Zhang et al., 2022). Notably, our initial bioinformatic screening did directly link KLFs to these genes, implying indirect inter-

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actions. These interactions could further explain both up- and down- regulatory effects, making further exploration imperative to better elucidate these mechanisms.

5. Conclusion

The heart is a vital organ that can be damaged by various factors, including stress, genetic defects, and environmental factors. At high dosage, isoproterenol can induce myocardial damage, leading to inflammation, loss of heart muscle cells, scarring, and remodeling or hypertrophy.

KLFs are involved in a variety of cellular processes, including gene transcription, cell growth, and differentiation. Our study showed that KLFs are dysregulated by myocardial damage, promoting the progression of abnormal hypertrophy. We showed that expression profiles of different KLFs change during different stages of cardiac damage, suggesting that KLFs may play a role in modulating the hypertrophic response and influencing downstream signaling pathways. The findings of this study provide new insights into the molecular mechanisms underlying cardiac damage and abnormal hypertrophy. A better understanding of their targets and interacting partners could help us lead to the development of new therapeutic interventions to treat heart diseases.

Supplementary Materials: The following supporting information can be downloaded at the website of this paper posted on Preprints.org. Supplemental Figure S1. Infarct Model. H&E staining A, B) 21d post infarct high fibroblast in-filtration, C) Loss of muscle fibers, D) Control no infarct. Supplemental Table S1. Primer list. Supplemental Table S2. Weights and lengths. Supplemental Table S3. Table of protein-protein interaction pairs from the STRING database.

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