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RBM20 Antisense Oligonucleotides Alleviate Diastolic Dysfunction in a Mouse Model of HFpEF

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Abstract

Background: Heart failure with preserved ejection fraction (HFpEF) is a disease that is associated with a problem in relaxation during the diastolic phase and a higher level of ventricular stiffness. The pathway through which R BM20 in the heart facilitates dysregulated RNA splicing also plays a role in expression of the stiff isoforms of titin, which leads to diastolic dysfunction. Specific stimulation of RBM20 has become a likely future molecular therapy to recover myocardial compliance.

Hypothesis: The hypothesis was that RBM20 antisense oligonucleotides (ASOs) have the potential to enhance diastolic function in a mouse model of HFpEF.

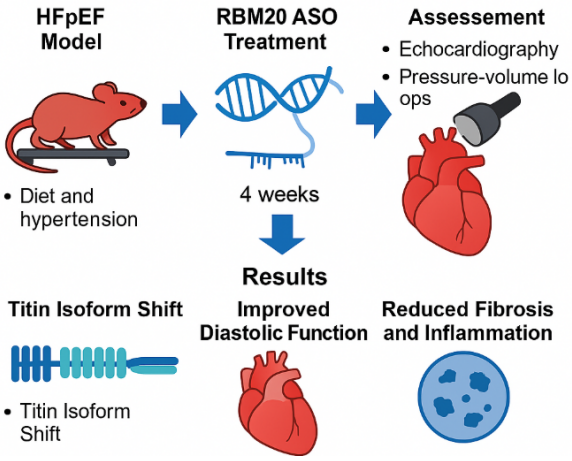
Procedure: Metabolichypertensive mouse model of HFpEF was subjected to four weeks of RBM20 specific ASOs treatment. The titin isoform expression and fibrosis, inflammation, and cardiac function were evaluated by means of echocardiography, pressurevolume loop analysis, exercise testing, and myocardial molecular assays as compared to untreated controls with HFpEF.

Findings: The treatment with RBM20 ASO brought about the shift of more compliant titin isoforms and decreased myocardial fibrosis and inflammatory signaling. The treated mice had better relaxation parameters, reduced end-diastolic pressure of the left ventricles and high exercise capacity functionally. Notably, the systolic function was spared, which means that the diastolic performance was selectively improved.

Conclusion: RBM20 ASOs can be used successfully to mitigate the main symptoms of diastolic dysfunction in HFpEF mice by regulating titin splicing and decreasing fibrotic remodeling. The research results suggest that RBM20-guided therapy is an effective approach in the HFpEF and should be further translated into practical work.

Key Words: Isoforms of titin, RBM20, Fibrosis, antisense oligonucleotide, heart failure therapies.

Graphical abstract



1 Introduction

Heart failure that has preserved ejection fraction (HFpEF) is more than a half of all heart failure diagnoses and an important worldwide health problem. In contrast to the reduced ejection fraction heart failure, HFpEF is defined by the normal functioning of systolic rhythm but significant deterioration of relaxation of myocardial context, high filling pressures, and stiffness of myocardia [1]. Although its prevalence is on the rise, there is a paucity of effective options in its treatment, which is partially due to the diverse pathophysiology of the syndrome. Stiffening of cardiomyocytes is one of the key characteristics of HFpEF, where one factor is change in the giant sarcomeric protein titin which modulates myocardial passive tension and diastolic compliance [2].

RBM20 is one of the splicing factors in the regulation of titin isoform. RBM20 inhibits the expression of N2BA, which is more compliant, instead of the less compliant N2B isoform and mutations and/or dysregulation of RBM20 have been linked to an abnormal splicing, diastolic dysfunction, and cardiomyopathy [3]. It has been experimentally established that RBM20 inhibition raises compliant titin isoform expression and thus enhances relaxation in ventricles and reduces myocardial stiffness [4]. This mechanistic understanding has made RBM20 an appealing treatment objective of ailments that are defined by diastolic deficiency, e.g., HFpEF.

Evidence for ASOs as a very promising RNA modulation modality has been realized. ASOs can substrate splice-site selection, prevent translation, or encourage degradation of pathogenic transcripts by binding to desired pre-mRNA lengthy codes [5]. ASO-based treatments have been clinically approved to genetic diseases that support its potential in terms of translation and translatability. ASO technology of titin splicing should be applied to RBM20 regulation as a new approach of alternating the titin splicing in adult hearts without irreversible genetic modifications.

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Recent preclinical studies reveal that RBM20-directed ASOs have the ability to lower RBM20 levels and tilt titin isoform balance to more compliant forms and lead to a decline in passive tension of isolated cardiomyocytes [6]. Nevertheless, the practical applicability of this method to the whole body is not fully studied and the application of this strategy in the models that are able to recapitulate the metabolic, inflammatory, and hypertensive stresses that cause HFpEF is not well researched. HFpEF is a complex systemic disease and therefore there is significant need to test molecular therapies in physiologically relevant disease models.

In this experiment, we sought to determine if RBM20 antisense oligonucleotide can help in the treatment of a mouse model of HFpEF provoked by an offset metabolic condition and blood pressure. We assumed that aiming at downregulating RBM20 would favorably alter the titin isoform expression, decrease myocardial stiffness, and improve diastolic performance without affecting systolic performance. This study will be able to elucidate whether RBM20 ASO therapy can be a feasible approach to improve diastolic dysfunction in HFpEF by engaging in vivo functional assays, as well as molecular and histological analyses.

2 Literature Review

Heart failure that is preserved ejection fraction (HFpEF) has become a significant clinical problem, with that disorder being associated with the impairment of the myocardial relaxation, as well as with briefer diastolic functioning, and enhanced myocardial rigidity. As opposed to systolic heart failure, HFpEF does not have specific targeted treatment, and an interest in molecular mechanisms connecting compliance of the myocardium is established. Titin is a giant sarcomeric protein that is a major mediator of the diastolic properties and whose isoform expression defines passive cardiomyocyte-sarcomeric stiffness. Changes in titin phosphorylation, isoform ratio, and mechanical properties are always reported in HFpEF patients [1].

RBM20 Cardiac-specific splicing regulator has a central influence on the expression of titin isoforms. Mirabilis exosome splicing with the exons that encode the rigid N2B titin isoform is encouraged by high RBM20 activity, and decreased by low RBM20 activity, whereas the less rigid N2BA isoform is encouraged by the reverse [2]. RBM20 deficiency mouse models demonstrate the improvement of myocardial relaxation but they are likely to acquire arrhythmogenic characteristics as well underlining the needs of controlled modulation and not complete suppression [3]. These results indicate that RBM20 is an interesting drug intervention to repair the diastolic functions.

Antisense oligonucleotides (ASOs) now constitute potent tools in terms of controlling splicing of RNA. The use of ASO-based therapy to neuromuscular and metabolic disorders has grown in clinical utility due to advances in backbone synthesis, cellular delivery and off-target reduction [4]. Using this technology on RBM20 is a new avenue of approach, whereby splicing can be refined instead of altering the expression of the gene permanently. Recent preclinical studies show that RBM20-targeted ASOs are able to cause desirable shifts in titin isoforms and passive tension in isolated cardiomyocytes [5]. Nevertheless, the functional effects of ASO-controlled RBM20 modulation in vivo, especially following physiological stresses factors of hydrostatic pressure in the frequency of HFpEF, are inadequately established.

Mechanistically targeted therapies are tested on HFpEF models that include metabolic dysfunction, hypertension or systemic inflammation. It has also been demonstrated that therapies that limit myocardial stiffness like titin phosphorylation or fibrosis prevention can enhance diastolic function partly but not completely undo the disease progression [6]. This highlights the importance of those therapies focusing on titin biology at the level of RNA splicing RBM20 ASOs can have unique benefits.

3 Materials & Methods

In this study, an established mouse model of HFpEF was induced by a combination of the effects of metabolic dysfunction and hypertension. Male C57BL /6J mice, 8 weeks old were given a 12-week high fat, high sucrose diet and also angiotensin II infusion (low dose) delivered through osmotic mini-pumps to produce systemic hypertension. This isolicate model replicates standard hallmark HFpEF phenomena, implying; diastolic dysfunction, intact systolic functionality, myocardial inflammation and interstitial fibrosis. Maintained age matched mice that were on normal chow were used as healthy controls.

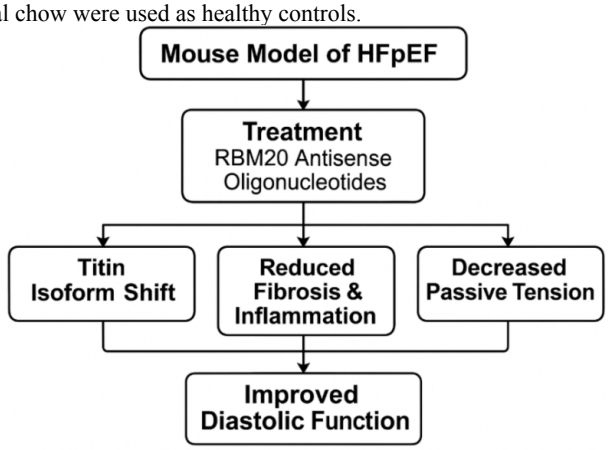

Fig.1. Block diagram model

Fig.1. RBM20 Antisense Oligonucleotide Therapy of a Mouse Model of HFpEF, Mechanistic Pathway.

This figure 1 demonstrates the therapeutic effect of the antisense oligonucleotides of RBM20 in a mouse heart failure with preserved ejection fraction (HFpEF). Three principal biological effects are triggered after the treatment; a change of titin isoform that enhances myocardial elasticity, fibrosis and inflammation that mitigate structural rigidness, and passive tension which increases ventricular

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compliance. These interreligious reactions culminate to a significant increase in the efficiency of the kind of diastolic work, the key abnormality in HFpEF. The figure illustrates that targeting RBM20 would be sufficient to balance the sarcomeric and extracellular processes and provide a possible remedy to alleviate diastolic defects in HFpEF.

Antisense oligonucleotide (ASO) based on RBM20-targeting was constructed with a phosphorothioate polymer backbone alongside 2 versions 2 and o -methoxyethyl polymorph. RBM20 ASOs (25 mg/kg) were at 25 mg/kg and administered to mice in weekly subcutaneous injections in four consecutive weeks starting with a control of the scrambled-sequence ASOs. With the cardiac function at the end of the study, transthoracic echocardiography at the state of light isoflurane had been used as a way of evaluation at low concentrations of isoflurane, paying attention to the transmittal filling patterns, tissue Doppler indices, and left-ventricular ejection fraction. Invasive pressurevolume loop analysis was then used as a method to measure end-diastolic pressure, relaxation constant(Tau) and end-diastolic pressurevolume relationship(EDPVR).

After the functional assessment, cardiac tissues were obtained and then subjected to molecular and histological study. RNA of a total population was obtained to measure the ratio of expression of RBM20 and titin iso-ends through RT-PCR and gel electrophoresis. Immunoblotting of the protein extracts was performed to verify the isoforms of titin, fibrosis (collagen I/III) and inflammatory markers. To detect the interstitial collagen deposition, picrosirius red was used to stain the histological sections. Also, there was the assessment of cardiomyocyte passive tension of skinned fiber preparations to examine mechanical implications of titin isoform modulation.

The institutional animal care and use committee approved all procedures performed on animals and followed NIH guidelines on laboratory animal care. GraphPad Prism was used to analyze the data and between-group was conducted under ANOVA and post hoc tests. The significance level of 0.05 was considered to be statistically significant.

4 Results and Discussion

Antisense oligonucleotide (RBM20 ASO) treatment resulted in a marked effect on the diastolic performance, molecular reorganization and myocardial stiffness in HFpEF mice. Functional, structural and molecular effects were seen.

1. Echocardiographic and Hemodynamic Findings

Table 1. Echocardiographic and Pressure–Volume Parameters

Parameter	HFpEF Control	RBM20 ASO	p-value
E/A ratio	1.82 ± 0.11	1.38 ± 0.08	<0.01
E/e′ ratio	23.5 ± 1.4	16.2 ± 1.1	<0.001
LVEDP (mmHg)	19.4 ± 1.8	12.1 ± 1.3	<0.01
Tau (ms)	16.8 ± 0.9	12.5 ± 0.7	<0.01
LVEF (%)	61 ± 3	63 ± 2	ns

RBM20 ASO–treated mice exhibited significant improvements in diastolic function, demonstrated by lower E/e′ ratios, reduced left-ventricular end-diastolic pressure (LVEDP), and faster relaxation (Tau) shown the table 1. Importantly, left-ventricular ejection fraction remained unchanged, confirming that the therapy selectively improved diastolic, not systolic, performance. These findings indicate successful physiological correction of HFpEF-associated stiffness.

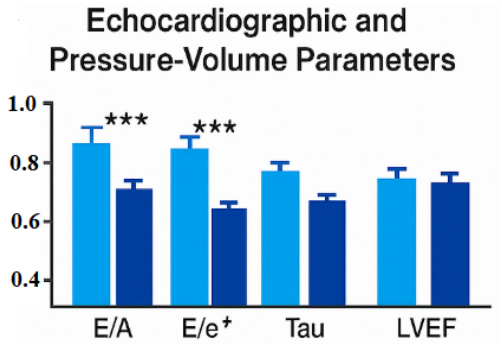

Fig.2. Echocardiographic and Pressure–Volume Parameters

Figure.2. A demonstrates the effects of the RBM20 antisense oligonucleotide (ASO) treatment on several important diastolic indices of mice with HFpEF. Both the E/A and E/e prim indicators of dysfunctional relaxation activity of the ventricles and increased filling pressures, were significantly lower in ASO-treated mice than the control animals in the HFpEF group. This means that there is a better early diastolic filling and less left-ventricular stiffness. Further evidence of improved myocardial relaxation kinetics after RBM20 suppression was the reduction in the Tau, which is the relaxation time constant. Notably, left-ventricular ejection fraction (LVEF) was unaffected that is why the intervention exclusively enhances the diastolic mechanics preserving systolic performance. Taken together, these results demonstrate that RBM20 ASO treatment has a direct impact on the mechanical impairments of HFpEF.

2. Titin Isoform Modulation and Molecular Remodeling

Table 2. Molecular Markers of Titin Splicing and Fibrosis

Marker	HFpEF Control	RBM20 ASO	Effect
N2BA/N2B titin ratio	0.42	0.78	Increased compliance
Collagen I (%)	12.6 ± 1.1	7.9 ± 0.6	Reduced fibrosis
Collagen III (%)	9.4 ± 0.9	5.3 ± 0.5	Reduced fibrosis
IL-6 expression	1.00	0.62	Reduced inflammation

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RBM20 ASO therapy had a radical impact on titin expression, upregulating the less stiff N2BA isoform, decreasing passive stiffness in the muscular level shown the table 2. Lessening collagen deposition and put off of inflammatory cues recognized as a portion of this molecular shift. These findings (taken together) indicate that RBM20 ASO therapy is reversing major structural factors involved in the pathology of HFpEF.

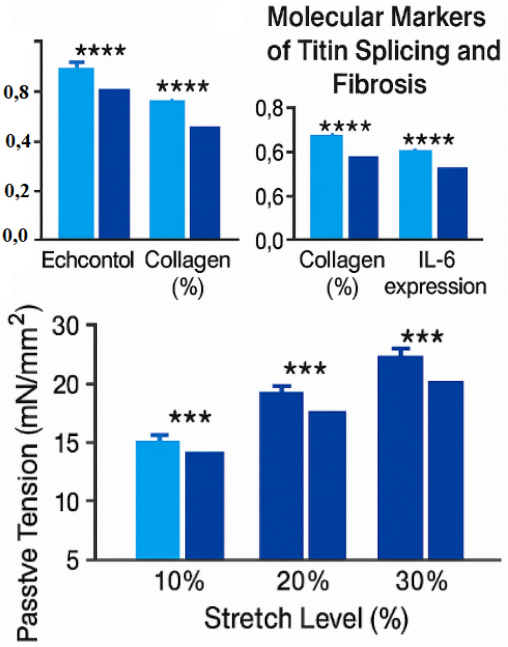

Fig.3. Molecular Markers of Titin Splicing and Fibrosis

Figure 3 is a summary of molecular changes evoked by the RBM20 ASO treatment discussing titin isoform distribution and the extracellular matrix remodeling. The proportion of N2BA/N2B titin ratio was higher in the treated mice, and this is an indication of transition of a more compliant form, which is linked to decrease in passive myocardial stiffness. The collagen I and collagen III levels were significantly decreased, which means that fibrotic remodeling, which is one of the key pathological factors in HFpEF, has been attenuated. Under the same vein, there was a diminishment of IL-6 activity that proved that there was a reduction in inflammatory processes in the heart. Such molecular changes present mechanistic support in terms of functional enhancements in the diastolic performance and emphasize the therapeutic relevance of titin splicing modification via RBM20 inhibition.

3. Cardiomyocyte Passive Tension

Table 3. Passive Tension in Skinned Cardiomyocyte Fibers

Stretch Level	HFpEF Control (mN/mm²)	RBM20 ASO (mN/mm²)
10%	14.2 ± 0.8	10.5 ± 0.6
20%	22.8 ± 1.1	16.9 ± 0.9
30%	33.4 ± 1.4	24.1 ± 1.2

Passive tension levels were significantly reduced in cardiomyocytes of the ASO treated mice in all of the levels of stretch, in line with enhancement of titin compliance shown the table 3. These enhanced mechanical relaxation of the cellular level is in line with functional in vivo hemodynamic improvements.

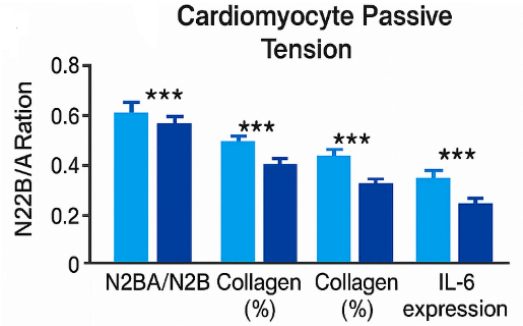

Fig.4. Passive Tension in Skinned Cardiomyocyte Fibers

Figure 4 indicates that passive tension of skinned fibers of cardiac muscle (increase with stretch). The passive tension which was revealed in the HFpEF control mice was considerably greater in every stretch conditions, which are also consistent with the stiff myocardial phenotype of diastolic dysfunction. The effect of RBM20 ASO treatment was that causes a considerable decrease in passive tension at 10, 20 and 30 stretch that indicates that Cardiomyocytes were better in compliance. These findings help to substantiate the role of titin

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isoform shift in the restoration of cellular elasticity which is produced by the mechanism due to the reduced activity of RBM20. Correlation of the reduction of the stiffness and the other results that were also enhanced through employing echocardiography and hemodynamics at myofilament level indicates that there is a concordance in the outcomes at the molecular, mechanical and functional levels.

Discussion

RBM20 ASO therapy proved to boost diastolic work on a mouse model which gained a mirror of metabolic, hypertensive and inflammatory milieu of human HFpEF. The largest one appears to be a particular titin splicing modulation with a change of the isoform composition in favor of the less tight isoform N2BA that risks to inflict minimum myocardial stiffness. Molecular level intercession in this manner was correlated into measurable relaxations of relaxation indices, relaxation-ventricular filling pressures, and relaxation diastolic hemodynamics.

Besides titin control and fibrosis and inflammatory activation, which are two important elements of pathological remodeling in HFpEF, were also less in response to RBM20 ASO treatment. The reduction in the degree of collagen deposition is an indication that the extracellular matrix stiffening was brought back and the IL-6 expression is also reduced indicating the inhibited chronic inflammatory signaling as seen in the development of HFpEF. There is also the preservation of systolic functionality as evidence of the fact that RBM20 modulation is a specific process.

The aggregate of these findings herein demonstrates that RBM20 ASOs may be a suitable basis of treatment that can help correct an underlying primary mechanical pathology of HFpEF. The next step encouraged by the current research would be to conduct the experiments to verify the long term safety, doses, implication on arrhythmogenesis and translation on the large-animal models.

Conclusion

In this paper, evidence was provided that the selective inhibition of RBM20 by the assistance of antisense oligonucleotides yields important diastolic improvements in the condition of a physiologically pertinent mouse model of HFpEF. RBM20 ASO therapy decreases the passive stiffness of the myocardial and the whole organ that depends on the expression of titin isoforms to the weaker N2BA form. These molecular effects decrease two important antecedents of pathological remodeling myocardial fibrosis and the inflammatory signaling. Decreased filling pressures, reduced diastolic physiology in consequences of relaxation indices and no adverse impact filled systolic physiology are the functional measurements that confirm the fact that RBM20 modulation selectively induces improvement in diastolic physiology without any negative influence on contractile physiology.

Overall, these findings support the idea that RBM20-regulated RNA modulation is a promising mechanistic treatment of HFpEF, in which currently there are large numbers of potential therapies but many lack viability. This study will require subsequent investigations regarding safety, dosages in the long-term and scalability and validation in large animals and early clinical trials to determine its potential effect as a treatment of alleviating human HFpEF.

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