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Integrating Galectin-3 Into a Computational Model of Cardiac Fibrosis Progression

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Undergraduate Honors Thesis

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I. Abstract

Cardiac fibrosis, a large contributor to heart failure, is the excessive accumulation of extracellular matrix in response to stress or injury. There are no approved treatments for cardiac fibrosis, and targeting specific species involved creates complex problems for drug development, so a computational model of the cardiac fibroblast signaling network can be used to observe the interactions involved in the progression of cardiac fibrosis. In this paper, a new protein called galectin-3 is integrated into this existing model, and connections are established to expand the coverage of the network. The additions are described, simulated using Netflux biological system simulation software, and assessed to determine the overall contribution galectin-3 has in the development of cardiac fibrosis. This addition will allow for new interactions to be studied, and galectin-3 will be a new target for potential therapies.

Keywords: *cardiac fibrosis, galectin-3, extracellular matrix, collagen, Netflux, simulation*

II. Introduction

II.A Cardiac Fibrosis

Heart failure affects approximately 6.2 million Americans, and one of the root causes of heart failure is an excessive accumulation of extracellular matrix (ECM), known as cardiac fibrosis [1]. The accumulation of ECM associated with cardiac fibrosis arises from the inability of damaged myocardium to regenerate after an injury, such as from a myocardial infarction (MI). The occurrence of an MI causes a loss of cardiomyocytes, and an inflammatory response is initiated to repair the damaged areas with scarring [2]. Excess buildup of the collagen-based scar

leads to increased stiffness of the heart, ultimately reducing left-ventricular ejection fraction. This disruption in the normal function of the heart increases the chance of disruptions, which can lead to heart failure over time [1].

Treatment options for cardiac fibrosis are very limited, as ECM scarring is crucial to the reparation of the heart after such injury to the cardiomyocytes. Without collagen repair, myocardial structural integrity would decrease significantly, ultimately increasing the susceptibility of the tissue to rupturing [3]. To conduct efficient studies of drug options and potential therapies for ECM accumulation, *in silico* simulation methods can be used, and such methods are used and developed in this study.

Computational methods of studying cardiac fibrosis development and treatment can be effective at determining complex relationships between involved species. While this method cannot replace *in vitro* and *in vivo* experimenting, it can extensively utilize the results produced from both, while effectively mitigating high costs and time-consuming individual studies [4]. This study assesses the addition of a pro-fibrotic protein, galectin-3, to a computational model of cardiac fibrosis.

II.B Galectin-3

Galectins are a family of carbohydrate-binding proteins with a high affinity for β-galactosides, and fifteen different galectins have been found in humans [5]. Three different structure types of galectins exist: prototype, tandem-repeat type, and chimeric type, and the only chimeric galectin is galectin-3, distinguishable by one C-terminal carbohydrate recognition domain (CRD) and a disordered N-terminal domain [5-7].

Galectin-3 has been previously known as Mac-2 antigen, IgE-binding protein, L-29, and CBP30, which stem from the multiple fields of studies that galectin-3 has been identified in, such as with macrophage population markers, kidney cells, leukemia development, and ECM formation [5]. Galectin-3 has been determined a pro-fibrotic species through monitoring fibroblast and macrophage activity with ECM accumulation around inflamed tissue, and it is suggested that galectin-3 activates the myofibroblasts associated with fibrotic activity [8]. Galectin-3's influence on fibrotic activity includes effects on epithelial mesenchymal transdifferentiation (EMT), cellular apoptosis, proliferation of myofibroblasts, and observable increases in ECM [8].

Galectin-3 has been considered a biomarker of cardiac fibrosis, as it is found to be highly upregulated in failed heart tissue when compared to functioning tissue [5,9]. When galectin-3 concentrations of heart failure patients were measured to compare rehospitalization and deaths, it was found that more deaths (43.2%) than rehospitalizations (28.4%) occurred when galectin-3 concentration was greater than 25.9 ng/mL, while concentrations between 20.0-25.9 ng/mL resulted in a greater rate of rehospitalization (35.1%) than death (28.4%), which suggests galectin-3 levels serve as a prognostic indicator of fibrosis and heart failure [9].

II.C Cardiac Fibroblast SNM with Netflux

A cardiac fibroblast signaling network model (SNM), using the open-source MATLAB-developed software package "Netflux" (https://github.com/saucermanlab/Netflux), produces dynamic computational models of pathways involved in the progression of cardiac fibrosis. The model uses logic-based differential equations to model species interaction, which are based on normalized Hill functions and controlled by logical AND, NOT, and OR operators [10]. Each

interaction is parameterized in Netflux by a reaction *weight*, which specifies the maximum activity of the interaction, an *n* value, which is the Hill coefficient parameter, and *EC50*, which is the half-maximal effective concentration parameter [11]. Netflux calculates the activation of species C by species A, specified as "A => C", using the described parameters and equations 1 and 2 below [12].

$$f_{act}(A) = \frac{A^n}{EC_{50}^n + A^n} \quad (1)$$

$$\frac{dC}{dt} = \frac{1}{\tau_D}(W_{AC}\, f_{act}(A)\, C_{max} - C) \quad (2)$$

The current model consists of 11 biochemical and biomechanical inputs, including angiotensin II (AngII), transforming growth factor beta (TGF-β), and norepinephrine (NE), and downstream of the inputs are 19 outputs, which include procollagens (proCI and proCIII), pro-matrix metalloproteinases (proMMPs), and tissue inhibitors of metalloproteinases (TIMPs). The model contains 201 interactions (edges) among 134 species (nodes) total.

III. Objective & Methods

The objective of this study is to integrate galectin-3 into the cardiac fibroblast SNM as an input species, and to connect the pro-fibrotic interactions of galectin-3 to existing species in the model. Model additions will be further assessed to determine the net contribution of galectin-3 to cardiac fibroblast activity.

A literature search will be conducted to confirm interactions between galectin-3 and multiple proteins, hormones, and cytokines that have been integrated into the existing SNM. Analysis and validation of additions will be performed through Netflux simulations and graphical illustration

of the effect of galectin-3 on the added species, as well as the output species (procollagens, proteinases, and TIMPs).

IV. Interactions

The first addition to the model is the activation of Galectin-3 by runt-related transcription factor 1 (RUNX1). RUNX1, activated by cAMP response-element binding protein (CREB), is critical to hematopoiesis, and it is associated with the survival of leukemia cells [13]. In a study involving human pituitary cells, it was found that the galectin-3 gene, LGALS3, contains two binding sites for RUNX1, and that RUNX1 further upregulates the expression of galectin-3 [14]. This would allow for the addition of the reaction “RUNX1 => Gal3fb” to represent the activation of galectin-3 feedback in the model when RUNX1 is present.

Beta-catenin, a protein involved in EMT, cell adhesion, and transcription of genes responsible for the Wnt pathway, is closely connected cancer progression when imbalanced [15]. In a study of incubated human hepatocellular carcinoma cells on the effects of galectin-3 on the beta-catenin pathway, it was observed that upregulation of galectin-3 increased beta-catenin signaling, which infers that beta-catenin depends on galectin-3 for activation [16]. Thus, the reaction "Gal3 => Bcatenin” was added to the model.

NADPH-oxidases (NOX) are reactive oxygen species that are activated upon a neutrophil’s interaction with an invasive species. [17] NOX has been identified as a contributor to cardiac fibrosis, and its relationship with galectin-3 has been studied. It has been discovered that galectin-3 is an activator of NOX in secreted neutrophils in a study of human skin legions [18]. This allowed for the reaction “Gal3 => NOX” to be added to the model.

Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has been studied as a contributor to fibrosis, as it up-regulates the expression of SNM outputs TIMP1 and osteopontin in the lungs of mice [19]. In a study of human lung cancer cells, it was found that while toll-like receptor 4 (TLR4) activates NF-κB, galectin-3 is a source of activation for TLR4, which then activates NF-κB [20]. Without the addition of TLR4, adding the activation of NF-κB by galectin-3 would be best accomplished by assuming a direct interaction. The reaction was then added to the model as "Gal3 => NFKB".

Ras GTPases are GTP-binding proteins that have multiple pathways involved with fibrotic and cancerous activity, such as the proliferation of myofibroblasts through the MAPK pathway [21]. A relationship of galectin-3 and Ras has been found that infers the activation of Ras by galectin-3, which ultimately activates mitogen-activated protein kinase kinase (MAPK) [22]. The reaction added for this finding was "Gal3 => Ras".

Transforming growth factor beta (TGF-β) is a known contributor to fibrotic activity, and it is active in the existing fibroblast model. It has been observed as one of the most central components of fibrosis development, as studies have shown that its signaling cascades lead to fibrotic tissue formation [23]. While galectin-3 and TGF-β appear to have similar functions, complex relationships have been found. A study on human bronchial cells confirmed that galectin-3 binds to the TGF-β receptor II (TGFBRII), which activates the expression of TGF-β [24]. However, the reaction added to the model utilizes the complex-forming activity of receptors TGFBRI and TGFBRII to propogate TGF-β signaling as "Gal3 => TGFBRI" [25].

Tumor necrosis factor alpha (TNF-α) is a proinflammatory cytokine that is known to contribute to adverse myocardial remodeling during an inflammatory response, as its primary responsibility is to regulate immune responses [26]. In a study of liver fibrosis using rats with

normal galectin-3 genes against rats with the galectin-3 gene inhibited, TNF-α was seen to have decreased when galectin-3 was inhibited, but when galectin-3 was active, TNF-α as well as collagen I and III content had increased [27]. This suggests the direct activation of TNF-α by galectin-3, and the reaction was added as “Gal3 => TNFa”.

The final addition to the model is the activation of galectin-3 by protein kinase C (PKC). Activation of PKC-α, the most common isoform of PKC, has been studied and is known to induce cardiomyocyte hypertrophy, which leads directly to heart failure [28]. A common study performed with galectin-3 is with one of PKC’s activators, AngII, where galectin-3 activity is increased significantly, as well as general pro-fibrotic activity [9,29]. However, further experimentation on rat cardiac tissue has targeted PKC-α as a potential activator of galectin-3, and it was found that through the inhibition of PKC-α, galectin-3 expression and collagen I content were decreased significantly [28]. This study allowed for the final reaction to be added to the model as “PKC => Gal3fb”

All new interactions involving galectin-3 in the fibroblast SNM are shown below in Table 1 with default weight, n, and EC50 listed.

Table 1. Fibroblast SNM Gal3 Additions

ID	Rule	Weight	n	EC50
i12	=> Gal3in	0.1	1.05	0.65
i24	Gal3in => Gal3	1.0	1.01	0.5
r189	Gal3fb => Gal3	0.5	1.01	0.5
r185	Gal3 => Bcatenin	1.0	1.05	0.65
r184	RUNX1 => Gal3fb	1.0	1.05	0.65
r190	Gal3 => NOX	1.0	1.05	0.5
r191	Gal3 => NFKB	1.0	1.05	0.5
r192	Gal3 => TGFB1R	1.0	1.05	0.5
r193	Gal3 => Ras	1.0	1.05	0.5
r194	Gal3 => TNFa	1.0	1.05	0.5
r195	PKC => Gal3fb	1.0	1.05	0.5

V. Results

The first observation made with the addition of galectin-3 is the procollagen (proCI and proCIII) activity when galectin-3 input is manipulated, which is shown below in Figure 1, where the input weight of galectin-3 was set to 0 (no input), 0.5, and 1.0 (maximum).

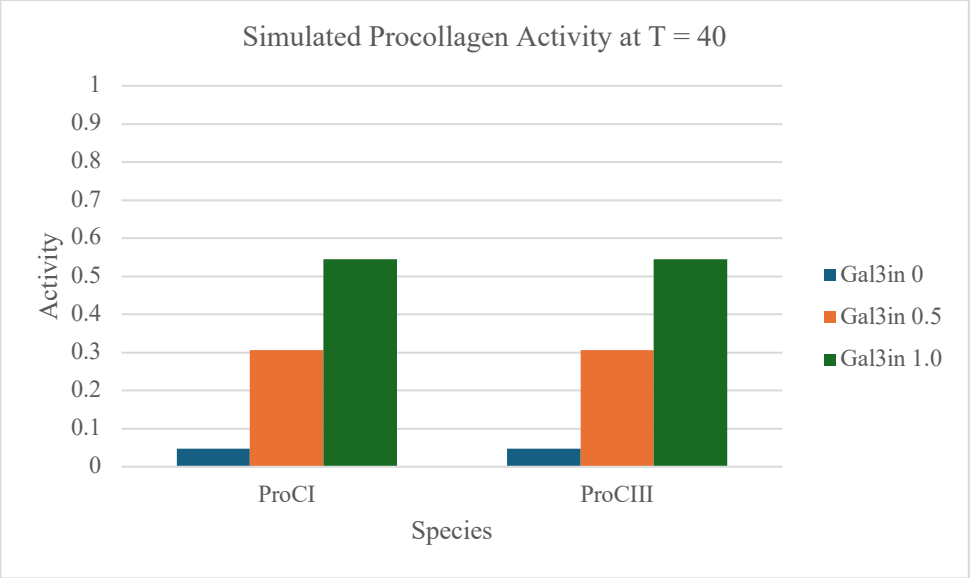

Figure 1. ProCI and ProCIII Activity with Varied Gal3 Input Weight

As expected, procollagen activity is increased as galectin-3 is introduced to the system at baseline activity (0.1 input weight) of all other input species. To show the change in activity with time while varying galectin-3 input weight, the activity of ProCI is plotted below in Figure 2.

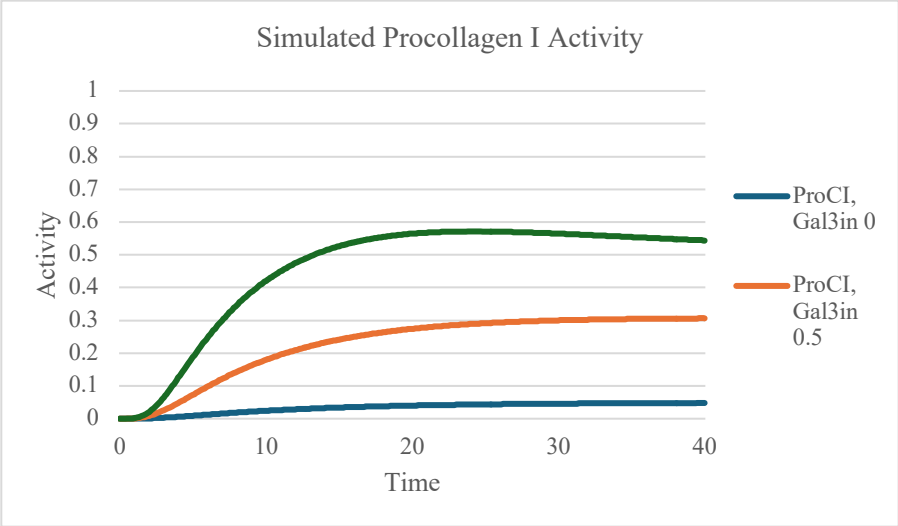

Figure 2. ProCI Activity with Varied Gal3 Input Weight

To analyze the activation of galectin-3 through the model, simulations were performed to mimic the study of AngII stimulation on mice with inhibition of the galectin-3 gene (Gal3KO, for knock-out) to compare the collagen volume fraction to mice with active galectin-3 [29]. In Netflux, the weights of all galectin-3 interactions were set to zero to simulate galectin-3 deactivation, and another simulation was created with galectin-3 interactions active, and the activity of ProCI was ultimately compared in both cases. Figure 3 shows the results of the experiment simulated in Netflux.

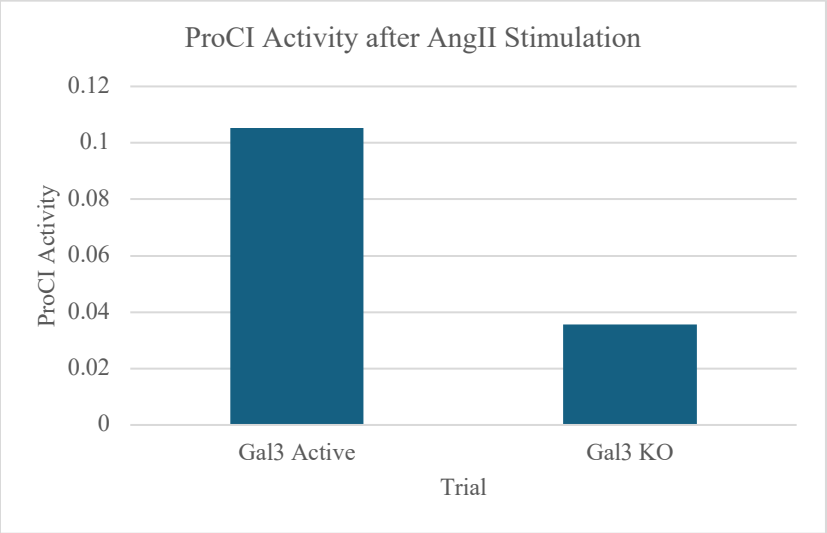

Figure 3. Netflux Simulation of ProCI Activity After AngII Stimulation

After stimulation with AngII in both studies on a system with active galectin-3, the collagen content, or ProCI activity, is observed to increase in both. When galectin-3 is deactivated, the collagen content is seen to decrease in both. This observation allows for the conclusion that the fibroblast SNM has been configured to properly activate galectin-3 in the case of AngII stimulation via PKC.

A plot of all downstream activations can be made while varying the input of upstream galectin-3, and such is shown in Figure 4 below.

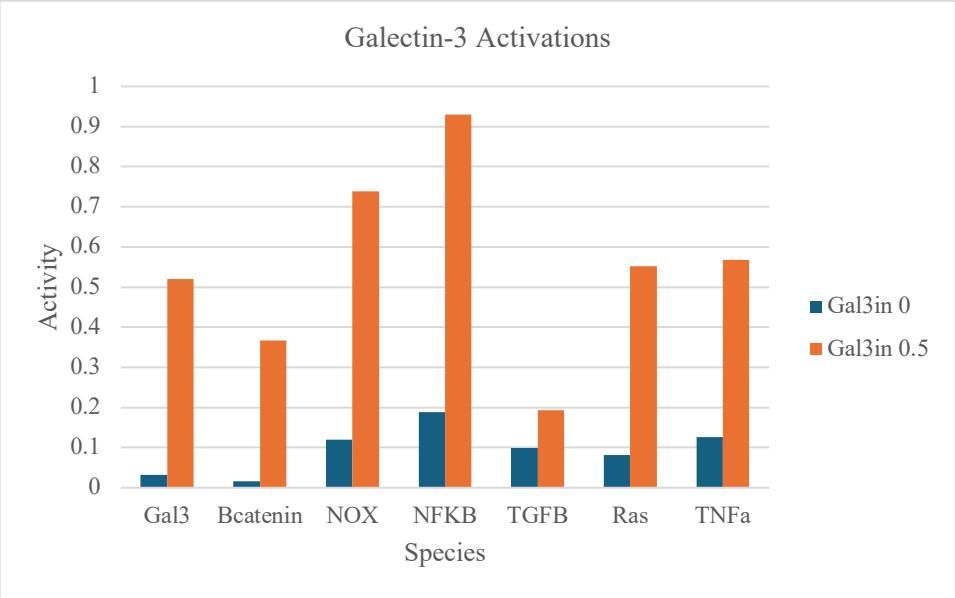

Figure 4. Activity of Downstream Activations by Galectin-3

Galectin-3 is included because while the input weight is zero, there is still activity seen by galectin-3 from upstream activations, since all other input weights are at 0.1 to simulate an environment that is under minimal stimulation. Figure 4 illustrates the sensitivity of these species to the addition of galectin-3, such as the extreme increase in activity by NF-κB.

Next, all model outputs are simulated to observe the effect of galectin-3 input on fibrotic activity. The fibroblast SNM outputs are compared with varying Gal3in, and the results at time 40 are shown below in Figure 5.

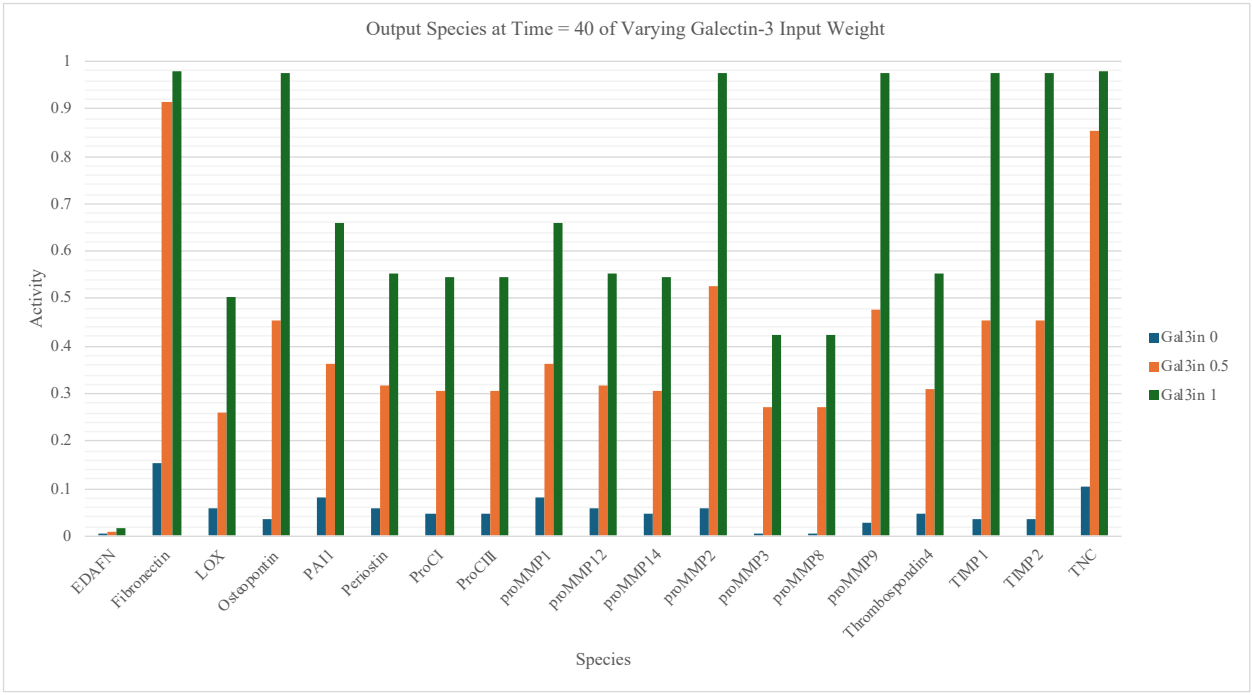

Figure 5. Fibroblast SNM Output Activity with Varied Gal3 Input

Figure 5 encompasses all pro-fibrotic and anti-fibrotic outputs that can be observed when studying cardiac fibrosis development. The anti-fibrotic species, proMMPs, are shown to trend upward with increasing galectin-3 input, with proMMP2 and proMMP9 exhibiting a high sensitivity to galectin-3 activity. This response of the proteinases is due to the natural reaction of the system to degrade the ECM accumulation, however, the sensitivity of TIMP1 and TIMP2 are observed to match the activity of proMMP2 and 9.

More sensitivities to galectin-3 are seen by matrix proteins fibronectin and tenascin-c (TNC), as they appear to approach maximum activity at only half maximum galectin-3 input. This sensitivity is expected, as both proteins are crucial to the deposition of ECM at the site of an injury, which would be signaled by galectin-3 [8,30].

To assess the overall effects of galectin-3 on cardiac fibroblast activity, a Matrix Content Score is calculated as $MCS = A_{matrix} + A_{protease} + A_{inhib}$, where $A_{matrix} = (ProCI + ProCIII +$

fibronectin + periostin + osteopontin + LOX)/6, $A_{protease}$ = (proMMP1 + proMMP2 + proMMP3 + proMMP8 + proMMP9 + proMMP12 + proMMP14)/7, and A_{inhib} = (TIMP1 + TIMP2 + PAI1)/3 [1]. The data in Figure 5 is used to calculate an MCS for each input weight of galectin-3, and Figure 6 below shows the net increase in matrix activity.

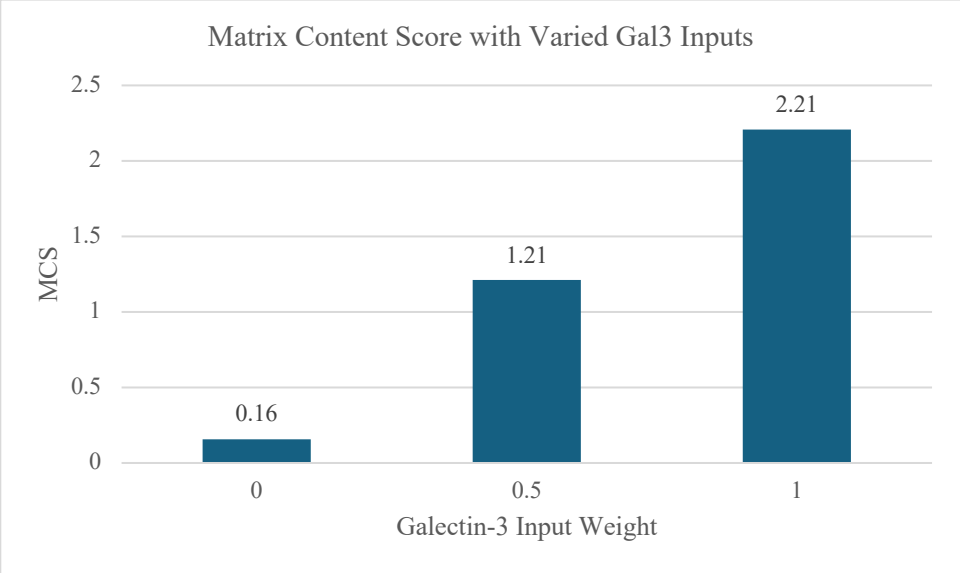

Figure 6. Matrix Content Scores

Similar to the method used to create Figures 5 and 6, the ECM output species can be simulated in a highly stimulated environment, such that all SNM input weights are set to 0.5. This was performed to create Figure 7 below, where galectin-3 input was varied to show the effect on the output species.

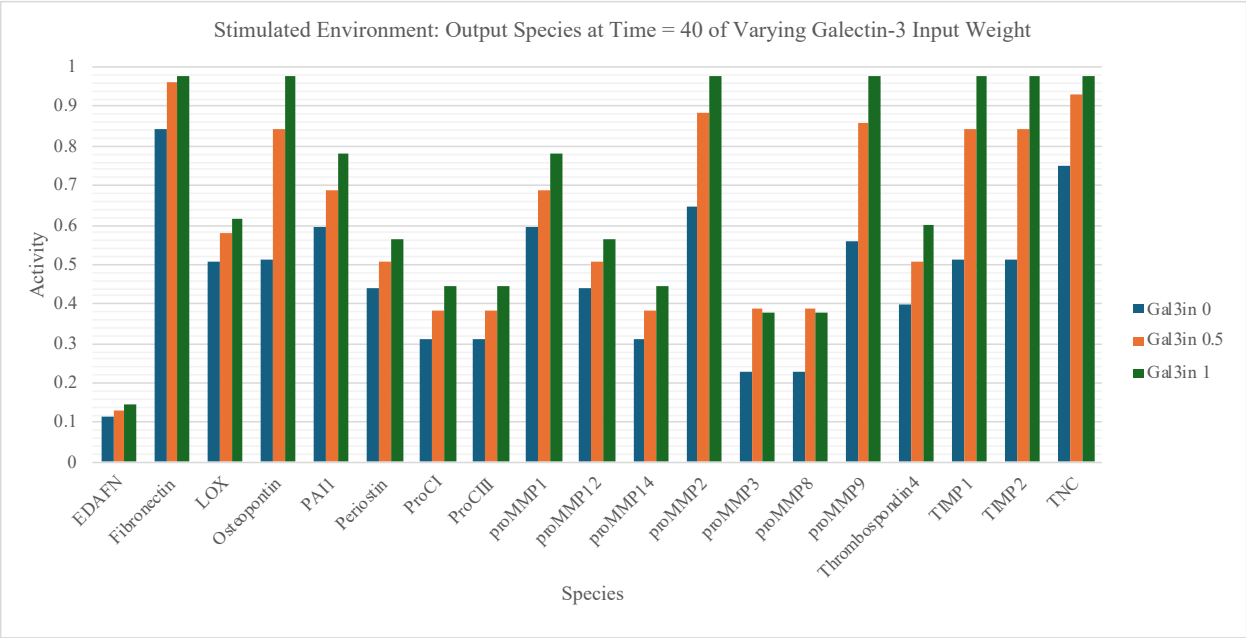

Figure 7. Fibroblast SNM Output Activity; Inputs at 0.5 Activity

With all inputs at 0.5 activity and galectin-3 input varied, there is still a significant increase in the activities of all species involved. To further observe the effects of galectin-3 in this case, the MCS is calculated as it was done previously in Figure 8 below, where a net MCS increase is observed with increasing galectin-3 input weight.

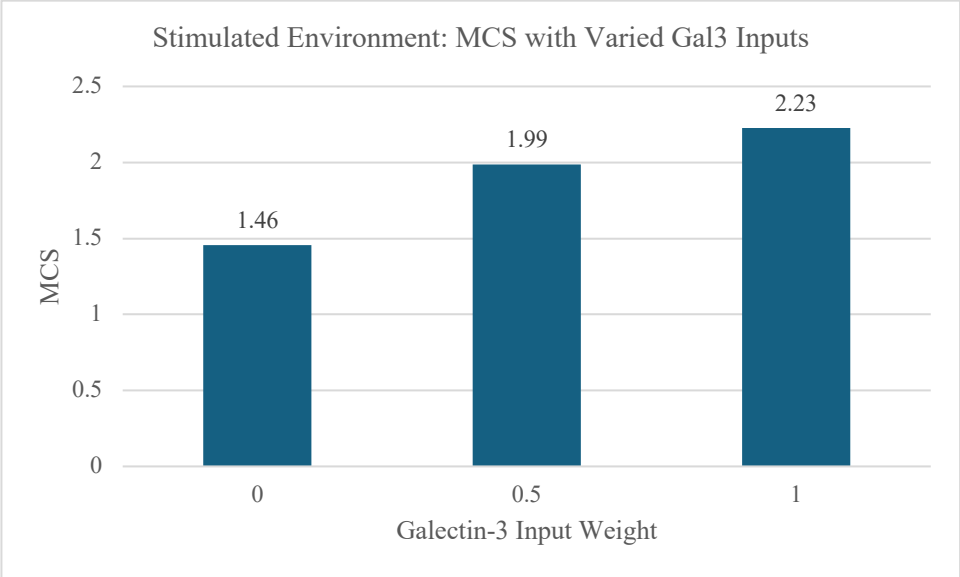

Figure 8. Matrix Content Scores of High-Activity Fibroblast Environment

VI. Conclusion & Future Direction

The addition of galectin-3 to the cardiac fibroblast SNM is one step forward in the study of cardiac fibrosis treatment, as it provides more pathways to understand and consider in the development of therapies and medications. This integration was successful in replicating the pro-fibrotic activity that galectin-3 exhibits in human and animal studies, with such replications illustrated by Figures 5 and 6. Galectin-3 addition, by itself, was simulated to raise ProCI and ProCIII activities by a factor of 5.38 at half-maximum input weight, and by a factor of 10.33 at maximum input weight, which trends consistently with studies done with galectin-3 stimulation.

The ability to target and monitor galectin-3 expression will provide new insight into how cardiac fibrosis and heart failure can be treated and prevented, however, there are limitations and more unknowns around the function of the protein. While there are relationships studied between galectin-3 and other species, exact mechanisms of all interactions have yet to be elucidated. With more studies done on galectin-3's function, more interactions may be discovered, further

expanding the understanding of the cardiac fibroblast network. With the many contributions of galectin-3, from normal cell function to the progression of multiple diseases, there is much more to uncover of the complete function of galectin-3.

VII. Acknowledgements

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VIII. References

[1] K. Watts, W. S. Nichols, and W. J. Richardson, "Computational screen for sex-specific drug effects in a cardiac fibroblast signaling network model," *Scientific Reports*, vol. 13, no. 1, Oct. 2023, doi: 10.1038/s41598-023-44440-9.

[2] P. Kong, P. Christia, and N. G. Frangogiannis, "The pathogenesis of cardiac fibrosis," *Cellular and Molecular Life Sciences*, vol. 71, no. 4, pp. 549–574, May 2013, doi: https://doi.org/10.1007/s00018-013-1349-6.

[3] F. G. Spinale, N. G. Frangogiannis, B. Hinz, J. W. Holmes, Z. Kassiri, and M. L. Lindsey, "Crossing Into the Next Frontier of Cardiac Extracellular Matrix Research," *Circulation Research*, vol. 119, no. 10, pp. 1040–1045, Oct. 2016, doi: https://doi.org/10.1161/circresaha.116.309916.

[4] K. Brunner, "Three Benefits of Using In Silico Technologies for Drug Discovery," *www.pharmasalmanac.com*. https://www.pharmasalmanac.com/articles/three-benefits-of-using-in-silico-technologies-for-drug-discovery

[5] N. Suthahar, W. C. Meijers, H. H. W. Silljé, J. E. Ho, F.-T. Liu, and R. A. de Boer, "Galectin-3 Activation and Inhibition in Heart Failure and Cardiovascular Disease: An Update," *Theranostics*, vol. 8, no. 3, pp. 593–609, 2018, doi: https://doi.org/10.7150/thno.22196.

[6] S. Sciacchitano *et al.*, "Galectin-3: One Molecule for an Alphabet of Diseases, from A to Z," *International Journal of Molecular Sciences*, vol. 19, no. 2, p. 379, Jan. 2018, doi: https://doi.org/10.3390/ijms19020379.

[7] T. Zhuang, H.-S. Lee, B. Imperiali, and J. H. Prestegard, "Structure determination of a Galectin-3-carbohydrate complex using paramagnetism-based NMR constraints," *Protein Science*, vol. 17, no. 7, pp. 1220–1231, Jul. 2008, doi: https://doi.org/10.1110/ps.034561.108.

[8] R. J. Slack, R. Mills, and A. C. Mackinnon, "The therapeutic potential of galectin-3 inhibition in fibrotic disease," *The International Journal of Biochemistry & Cell Biology*, vol. 130, p. 105881, Jan. 2021, doi: https://doi.org/10.1016/j.biocel.2020.105881.

[9] Labroots, "Peter McCullough - Galectin 3 A step towards a clearer pathway in heart failure management," *YouTube*. Sep. 22, 2015. [Online]. Available: https://www.youtube.com/watch?v=TeLvk6raZoM

[10] M. J. Kraeutler, A. R. Soltis, and J. J. Saucerman, "Modeling cardiac β-adrenergic signaling with normalized-Hill differential equations: comparison with a biochemical model," *BMC systems biology*, vol. 4, no. 1, Nov. 2010, doi: https://doi.org/10.1186/1752-0509-4-157.

[11] J. Saucerman, "Netflux Documentation," 3AD. Available: https://github.com/saucermanlab/Netflux/blob/master/Netflux/Netflux%20Documentation.docx

[12] J. Saucerman, "Introduction to Netflux," *www.youtube.com*. https://www.youtube.com/watch?v=tU2gU8d5hvQ&t=204s

[13] R. Sood, Y. Kamikubo, and P. Liu, "Role of RUNX1 in hematological malignancies," *Blood*, vol. 129, no. 15, pp. 2070–2082, Apr. 2017, doi: https://doi.org/10.1182/blood-2016-10-687830.

[14] He Yu Zhang *et al.*, "RUNX1 and RUNX2 upregulate Galectin-3 expression in human pituitary tumors," *Endocrine*, vol. 35, no. 1, pp. 101–111, Nov. 2008, doi: https://doi.org/10.1007/s12020-008-9129-z.

[15] T. Valenta, G. Hausmann, and K. Basler, "The many faces and functions of β-catenin," *The EMBO Journal*, vol. 31, no. 12, pp. 2714–2736, May 2012, doi: https://doi.org/10.1038/emboj.2012.150.

[16] M. Song *et al.*, "Galectin-3 favours tumour metastasis via the activation of β-catenin signalling in hepatocellular carcinoma," vol. 123, no. 10, pp. 1521–1534, Aug. 2020, doi: https://doi.org/10.1038/s41416-020-1022-4.

[17] A. Cave, D. Grieve, S. Johar, M. Zhang, and A. M. Shah, "NADPH oxidase-derived reactive oxygen species in cardiac pathophysiology," *Philosophical Transactions of the Royal Society B: Biological Sciences*, vol. 360, no. 1464, pp. 2327–2334, Nov. 2005, doi: https://doi.org/10.1098/rstb.2005.1772.

[18] A. Karlsson, P. Follin, H. Leffler, and C. Dahlgren, "Galectin-3 Activates the NADPH-Oxidase in Exudated but not Peripheral Blood Neutrophils," *Blood*, vol. 91, no. 9, pp. 3430–3438, May 1998, doi: https://doi.org/10.1182/blood.v91.9.3430.

[19] J. Dong and Q. Ma, "In vivo activation and Pro-Fibrotic function of NF-KB in fibroblastic cells during pulmonary inflammation and fibrosis induced by carbon nanotubes," *Frontiers in Pharmacology*, vol. 10, Oct. 2019, doi: 10.3389/fphar.2019.01140.

[20] W. Zhou, X. Chen, Q. Hu, X. Chen, Y. Chen, and L. Huang, "Galectin-3 activates TLR4/NF-κB signaling to promote lung adenocarcinoma cell proliferation through

activating lncRNA-NEAT1 expression," *BMC Cancer*, vol. 18, no. 1, May 2018, doi: https://doi.org/10.1186/s12885-018-4461-z.

[21] J. M. Muñoz-Félix and C. Martínez-Salgado, "Dissecting the Involvement of Ras GTPases in Kidney Fibrosis," *Genes*, vol. 12, no. 6, p. 800, May 2021, doi: https://doi.org/10.3390/genes12060800.

[22] P. P. Ruvolo, "Galectin 3 as a guardian of the tumor microenvironment," *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*, vol. 1863, no. 3, pp. 427–437, Mar. 2016, doi: https://doi.org/10.1016/j.bbamcr.2015.08.008.

[23] N. G. Frangogiannis, "Transforming growth factor–β in tissue fibrosis," *Journal of Experimental Medicine*, vol. 217, no. 3, Feb. 2020, doi: https://doi.org/10.1084/jem.20190103.

[24] J. F. Calver *et al.*, "Defining the mechanism of galectin-3-mediated TGF-β1 activation and its role in lung fibrosis," *The Journal of Biological Chemistry*, p. 107300, Apr. 2024, doi: https://doi.org/10.1016/j.jbc.2024.107300.

[25] N. Ni, X. Fang, and Q. Li, "Functional similarity between TGF-beta type 2 and type 1 receptors in the female reproductive tract," *Scientific Reports*, vol. 11, no. 1, Apr. 2021, doi: https://doi.org/10.1038/s41598-021-88673-y.

[26] M. Sun *et al.*, "Tumor Necrosis Factor-α Mediates Cardiac Remodeling and Ventricular Dysfunction After Pressure Overload State," *Circulation*, vol. 115, no. 11, pp. 1398–1407, Mar. 2007, doi: https://doi.org/10.1161/circulationaha.106.643585.

[27] Ki Tae Yoon, H. Liu, J. Zhang, S. Han, and S. S. Lee, "Galectin-3 inhibits cardiac contractility via a tumor necrosis factor alpha-dependent mechanism in cirrhotic

rats," *Clinical and molecular hepatology*, vol. 28, no. 2, pp. 232–241, Apr. 2022, doi: https://doi.org/10.3350/cmh.2021.0141.

[28] X. Song *et al.*, "Protein kinase C promotes cardiac fibrosis and heart failure by modulating galectin-3 expression," *Biochimica Et Biophysica Acta. Molecular Cell Research*, vol. 1853, no. 2, pp. 513–521, Feb. 2015, doi: 10.1016/j.bbamcr.2014.12.001.

[29] Germán Esteban González *et al.*, "Cardiac-deleterious role of galectin-3 in chronic angiotensin II-induced hypertension," *American Journal of Physiology-heart and Circulatory Physiology*, vol. 311, no. 5, pp. H1287–H1296, Nov. 2016, doi: https://doi.org/10.1152/ajpheart.00096.2016.

[30] C. Frantz, K. M. Stewart, and V. M. Weaver, "The Extracellular Matrix at a Glance," *Journal of Cell Science*, vol. 123, no. 24, pp. 4195–4200, Dec. 2010, doi: https://doi.org/10.1242/jcs.023820.