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A Computational Model of the Pulmonary Fibroblast Signaling Network as Related to Idiopathic Pulmonary Fibrosis

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A COMPUTATIONAL MODEL OF THE PULMONARY FIBROBLAST SIGNALING NETWORK AS RELATED TO IDIOPATHIC PULMONARY FIBROSIS

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ABSTRACT

Idiopathic pulmonary fibrosis, or IPF for short, is an interstitial lung disease that primarily affects the interstitium of the lungs (the tissue around the alveolar space). Overtime this disease causes scar tissue to form in these tissues, making them fibrous and stiff. As the disease progresses the lungs lose their ability to function properly, preventing the adequate intake and distribution of oxygen. There is currently no known cause for IPF and furthermore there are few treatments available. Medications such as pirfenidone and n intedanib can be used to slow the progression of the disease and can be used along with other drugs to mitigate symptoms but there is no cure. With the typical lifespan prognosis being 2-5 years from diagnosis and there being few options for treatment, the development and distribution of new and more effective treatments for IPF is critical.

A group at the Medical University of South Carolina (MUSC) has recently discovered a drug that has promising results for its use in treating IPF. However, the mechanism of this drug is unknown to its developers. Understanding a drugs mechanism can be key in refining its dosage, combination with other drugs, etc. in order to ultimately produce the most effective treatment. With the enormous number of possible molecular targets within cells there are far too many possibilities for the mechanism of this drug to do physical experiments for each cellular signaling molecule. In order to lessen this issue, we have developed a computational model of the cellular signaling pathways in pulmonary fibroblasts (the cells primarily responsible for the creation of the fibrous scar

tissue). We particularly focused on those signaling molecules and pathways that have previously been linked to fibrosis both within lung tissue and other bodily tissue.

The resulting pulmonary fibroblast signaling network model contains 111 signaling molecules that participate in 161 individual reactions. Validation procedures show that the model predicts with 74.4% accuracy when compared to literature data. Sensitivity analysis simulations were performed in order to further characterize the model, allowing for the identification of the most sensitive and the most influential nodes in the model. Further simulations were performed in order to make predictions as to the possible target mechanism of the preliminary MUSC IPF drug.

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CHAPTER ONE

INTRODUCTION

Idiopathic pulmonary fibrosis, or IPF, is a progressive lung disease characterized by excessive scarring of the pulmonary interstitium (tissue surrounding the alveolar space) after some initial injury has occurred [1]. This scarring is primarily caused by the excessive buildup of extracellular matrix (ECM) proteins, particularly collagens. The development of this fibrous scar tissue prevents the lungs from functioning properly, thus preventing the proper intake and distribution of oxygen into the bloodstream. Lack of adequate oxygen levels in tissues, referred to as hypoxia, can lead to a variety of supplemental pathologies.

IPF is of particular concern among interstitial lung diseases due to its exceptionally poor prognoses as it typically leads to death within 2-5 years of diagnosis [2]. IPF is estimated to affect 100,000 Americans with somewhere between 30,000 and 40,000 new cases each year [3]. The cause of IPF is still unknown, although some correlating factors have been identified. Sex seems to play a role with approximately 75% of people affected by IPF being male. Another seemingly significant factor is a patients age. Nearly all cases of IPF occur in patients over the age of 50 with increasing incidence along with increasing age.

There is currently no cure for IPF. Treatment options for IPF patients include antifibrotic drugs such as pirfenidone (more commonly known as Esbriet) and nintedanib (more commonly known as Ofev). Both drugs have shown positive results in slowing the

progression of IPF, however they do not reverse any damage that has already been done nor do they treat the various symptoms caused by IPF such as breathlessness, chronic cough, fatigue, etc. [2]. These anti-fibrotic medications also come with side effects that prevent some patients from being able to utilize them.

There is a small variety of other options for patients with IPF in order to address the symptoms of the disease. Corticosteroids, such as prednisone, are anti-inflammatory drugs that work by suppressing a patient's immune system in order to improve IPF symptoms [1]. The use of corticosteroids, however, has been shown to exacerbate the condition in patients with stable scarring that is initially progressing slowly. Thus, this treatment option, like the anti-fibrotic medications mentioned prior, is not available to all IPF patients. Another immunosuppressive drug called azathioprine has also been tested for use in IPF patients both alone and in conjunction with corticosteroids but there has been inconclusive evidence as to its efficacy in this application [4]. The most drastic and invasive form of treatment for IPF patients is a lung transplant which is generally much more effective in improving both the patient's quality and length of life compared to other previously mentioned treatments. However, like with all organ transplants, the number of donor lungs is very limited and thus many patients never have this treatment option.

IPF has proven very difficult to treat and study due to the fact that it is a very complex disease. There are many different signaling molecules involved its pathogenesis. Aside from the large number of factors involved, these factors can also change roles based on the level in which they are present. Not only is the initial cause of this disease

still a mystery but its progression into later stages is also not very well understood. The combination of these two unknowns makes it exceedingly more challenging to develop effective treatments.

With so many possible factors affecting the development and progression of IPF, one avenue of research that can be beneficial in handling so many possibilities is the use of computational modeling. Computational models have the ability to integrate many influencing components that would otherwise be difficult to intuitively predict. They can also compute scenarios in a more sensitive manor, allowing researchers to obtain results that they otherwise could not due to shortcomings with measuring techniques or the lack of statistical significance between replicate trials. There is currently a great shortage of computational models applicable to IPF research. We, therefore, have added to this research gap by constructing a computational model of the signaling networks of pulmonary fibroblasts that can be applied in multiple ways such as in predicting possible drug targets or combination targets in order to optimize the efficacy of present and future treatment options.

CHAPTER TWO

METHODS

Model Development

A molecular signaling network for pulmonary fibroblasts was manually constructed using data collected from previous experimental studies found in literature. A literature review of the molecular signaling in cardiac fibroblasts was previously conducted in order to construct a signaling network model for cardiac fibroblasts [5]. This review resulted in the creation of a network for cardiac fibroblasts focused on the following pathways: AngII angiotensin II), TGFβ (transforming growth factor β1), mechanical stimulation (in tension), IL6 (interleukin 6), IL1 (interleukin 1), TNFα (tissue necrosis factor α), NE (norepinephrine), PDGF (platelet derived growth factor), ET1 (endothelin 1), NP (natriuretic peptide), and forskolin. These inputs are unique biochemical or mechanical stimuli that are modified in fibrosis pathogenesis, including IPF. This initial signaling network consisted of 91 individual signaling molecules that are involved in 131 individual reactions.

A literature review of molecular signaling in pulmonary fibroblasts was conducted with a focus on the following pathways: FGF (fibroblast growth factor), IGF1 (insulin-like growth factor 1), IGF2 (insulin-like growth factor 2), S1P (sphingosine-1-phosphate), and LPA (lysophosphatidic acid) [6-37]. These pathways were explored as they are commonly-accepted to play roles in IPF. The literature review was conducted by searching for full reaction pathways as well as individual reactions directly upstream or

downstream of each specific molecule. The results of this pulmonary fibroblast literature review were used to make additions to the previously constructed cardiac fibroblast model in order to make it more representative of pulmonary fibroblasts. Studies that focused on specific signaling mechanisms were used in the creation of the network structure. Studies using multiple cell types were used in this part of the literature review, though the majority were various types of fibroblasts. This was due to a lack of studies specifically on pulmonary fibroblasts.

Species and reaction dynamics were computed by utilizing a normalized-Hill differential equation approach in conjunction with the AND and OR Boolean operators. Each species is modeled by its own corresponding differential equation, which computes activation levels in fractional units. The default species parameters used include y_{init} (0), y_{max} (1), and the time constant (τ). The time constant is set at 1 hour for all present additions to the model. However, τ values differ in the initial cardiac fibroblast model (0.1hr, 1hr, or 10hrs depending on the reaction type). Default reaction parameters further include weight (1), the Hill coefficient (1.4), and EC50 (0.6). A Microsoft Excel database detailing all species and reactions in the model that was initially created during the construction of the cardiac fibroblast model was expanded to include the new species and reactions identified for pulmonary fibroblasts. The system of ODEs representing the model was then generated by using this Excel file and the open-source Netflux software available at: https://github.com/saucermanlab/Netflux, and executed in MATLAB. The following equations are the normalized Hill equations and equations for AND and OR Boolean operators that Netflux uses to construct individual species differential equations.

In these equations n is the hill coefficient parameter, EC50 is the half-maximal effective concentration parameter, and B is a coefficient of the relationship between the parameters n and EC50.

$$f_{activate}(X) = \frac{BX^n}{K^n + X^n}$$

$$f_{inhibit}(X) = 1 - \frac{BX^n}{K^n + X^n}$$

$$B = \frac{EC_{50}^n - 1}{2EC_{50}^n - 1}$$

$$K = (B - 1)^{1/n}$$

$$output(AND) = f(x) * f(y)$$

$$output(OR) = f(x) + f(y) - f(x) * f(y)$$

Model Validation

A further literature review was conducted for the validation of the constructed network [30, 33, 38-58]. Reference studies were found by searching combinations of each input and output of the model along with the following phrases: "pulmonary fibrosis", "pulmonary fibroblast", "lung fibroblast" (e.g. "TGFβ effect on collagen pulmonary fibroblast"). Only studies that were performed using pulmonary fibroblasts were used in the validation of the model. Two of the articles used in validation were also referenced when constructing the model, all other validations references are separate

from those used for model construction. The validation of the signaling network model was completed by comparing the change in activity (increase, decrease, or no change) of the model simulations with experimental data. Model simulation results were obtained by first running a baseline simulation in which all input reactions were set at a weight of 0.25 (i.e., 25% activation). Simulations were then run one at a time with the input reaction weights set at 1 (100% activation). These two sets of results were then compared in order to determine the change in activity that the model predicts when being positively treated with the input signaling molecules. Differences of less than 0.1% between the baseline values and positive treatment values from model simulations were considered "no change". All simulations were run for 100 seconds in order for outputs to reach their steady-state values.

Sensitivity Analysis

A blocking treatment sensitivity analysis and a positive treatment sensitivity analysis were both performed on the network. The blocking treatment analysis was performed by individually blocking each node one at a time and measuring the resulting change in activity levels of all other nodes. A baseline blocking control simulation was first performed by setting all input reaction weights to 0.5 (50% activation) and then recording the activity levels of all nodes. Following this baseline simulation, 111 simulations were performed in which each single species activity was blocked one at a time. This was achieved by setting the initial and maximum activity levels (y_{init} and y_{max})

of each node to 0, and setting each individual differential equation equal to 0 (e.g. dydt(AngII) = 0). The change in activity levels were then calculated from these two data sets, the baseline data and the blocking treatment data. This was done by subtracting the baseline activity levels from the blocked treatment data activity levels.

The positive treatment sensitivity analysis was likewise performed by individually treating with one species at a time and measuring the resulting change in activity levels of all other nodes. A separate baseline positive treatment control simulation was first performed by setting all input reaction weights to 0.25 and then recording the activity levels of all of the nodes. Following this baseline simulation, 111 simulations were performed in which a single species activity was increased at a time. This was done by setting the initial activity level (y_{init}) of each node equal to 1. The change in activity levels were then calculated from these two data sets, the baseline data and the positive treatment data. This was done by subtracting the baseline activity levels from the positive treatment activity levels. All simulations were run for 100 seconds in order for outputs to reach their steady-state values.

Each set of activity change data was further analyzed in order to identify the most influential and the most sensitive nodes in the model for both blocking and positive treatment scenarios. The most influential nodes were identified by summing the absolute values of the changes in activity of all outputs per single input. The input species that had the greatest sum of the absolute values of the changes in activity of outputs were determined to be the most influential nodes in the network. The most sensitive nodes were identified by summing the absolute values of the changes in activity of all inputs per

single output. The output species that had the greatest sum of the absolute values of the changes in activity of inputs were determined to be the most sensitive nodes in the network.

MUSC IPF Drug Target Analysis

A group of researchers at the Medical University of South Carolina (MUSC) recently discovered a drug that has shown promising results in the treatment of IPF. However, the mechanism by which this drug acts to treat IPF is as of yet unknown. An analysis of the network was performed in order to identify possible targets of this MUSC IPF drug. Because little is known about the mechanism by which MUSC's preliminary IPF drug acts, there is a possibility that it could act either as an antagonist (blocking the activity of a signaling molecule) or an agonist (increasing the activity of a signaling molecule). For this reason, similar to the way in which the sensitivity analysis was performed, both positive treatment and blocking treatment simulations were performed.

The baseline simulation for blocking treatments was performed with the weights of all input reactions set to 0.5. The blocking treatment simulations were then performed by individually blocking each species by setting each individual species initial activity level and maximum activity level to 0, and setting each individual differential equation equal to 0 (e.g. dydt(AngII) = 0). The baseline simulation for positive treatments was performed with the weights of all input reactions set to 0.25. The positive treatment simulation was then performed by individually treating with each species in the network

by setting individual species initial activity level to 1. All simulations were run for 100 seconds in order for outputs to reach their steady-state values.

The change in activity between corresponding baseline and treatment data sets was then calculated by subtracting the baseline data from the treatment data. This allowed for the determination of whether specific treatments resulting in an increase, decrease, or no change in activity. This data was then compared to experimental results from the researchers at MUSC detailing how the IPF drug affects specific genes and signaling molecules. The species included in both data sets and thus those able to be compared include AngII, αSMA, TIMP1, PAI1, proMMP1, CImRNA, and CIIImRNA. IL1 and TNFα are also included in both the MUSC IPF drug profile data and in the model; however, these signaling molecules are only inputs for the model. Since there is nothing upstream of these species in the model they are only affected by manual inputs and as such are not used in the analysis for predicting possible drug targets of the MUSC IPF drug.

The MUSCS IPF drug caused the following change in activity/expression results for these outputs/signaling molecules: AngII – no change, αSMA – decrease, TIMP1 – decrease, PAI1 – decrease, proMMP1 – increase, CImRNA – decrease, CIIImRNA – decrease. AngII is the only identified molecule that was found to have no change in activity with MUSC IPF drug treatment. Changes in simulated activity levels of less than 1% were considered no change for AngII. This was not applied to all simulated changes in activity levels due to the fact that computational models have the ability to detect

smaller changes than can be measured in physical experiments or replicated in physical experiments with statistical significance.

In order to identify the signaling molecules that are most likely the targets of the MUSC IPF drug, each treatment scenario (blocking and positive treatment) was analyzed to see if they correctly matched the drug treatment profile. Once all scenarios that correctly matched the drug profile were identified, the sum of the absolute values of the changes in activities (not including AngII data) were found. The species that correctly matched the MUSC IPF drug profile and had the largest sum of the absolute values of the changes in activity were determined to be the most likely targets of the MUSC IPD drug.

CHAPTER THREE

RESULTS

A Computational Model of Pulmonary Fibroblast Signaling

After performing the pulmonary fibroblast literature review as described previously, 19 new signaling molecules and 34 new reactions were added to the model. Of the 34 reactions added, 5 were input reactions (FGF, IGF1, IGF2, S1P, and LPA). Thus, after these model additions the network consists of 111 signaling molecules that interact in 161 reactions (not including inputs) as can be seen in Figure 1. Of the 111 total species, 14 are outputs including CImRNA (collagen 1 mRNA), CIIImRNA (collagen 3 mRNA), periostin, fibronectin, EDAFN (extra domain A of fibronectin), proMMP1 (precursor to matrix metalloproteinase 1), proMMP2 (precursor to matrix metalloproteinase 2), proMMP9 (precursor to matrix metalloproteinase 9), proMMP14 (precursor to matrix metalloproteinase 14), PAI1 (plasminogen activator inhibitor 1), TIMP1 (tissue inhibitor of matrix metalloproteinase 1), TIMP2 (tissue inhibitor of matrix metalloproteinase 2), proliferation, migration, and αSMA (alpha smooth muscle actin). These are the main species that are focused on when performing model simulations.

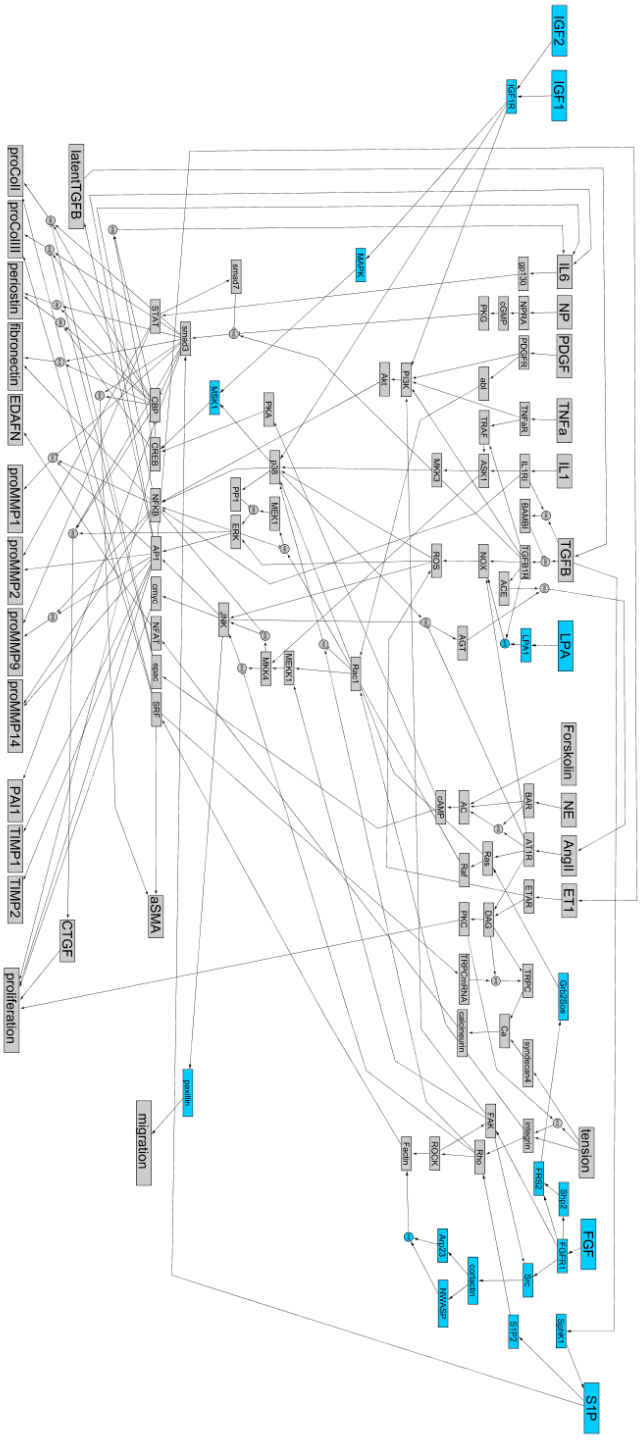

Figure 1: Schematic of the pulmonary fibroblast signaling network model; grey boxes indicate the cardiac fibroblast model; blue boxes indicate new nodes added for the pulmonary fibroblast model

Model Validation

The network model was validated using 39 input-output relationships found from 24 experimental studies. The model accurately predicts 29 out of 39 input-output relationships (74.4% accuracy). Contradictory information was found in regard to 4 additional input-output relationships and as such these were not included in the model accuracy calculation. These 4 contradictory cases include input-output relationships between IL6-proliferation, PDGF-C1mNRA, TNFα-proliferation, and FGF-C1mRNA. Figure 2 summarizes the validation results, comparing the predicted change in activity of outputs to the change reported in published literature.

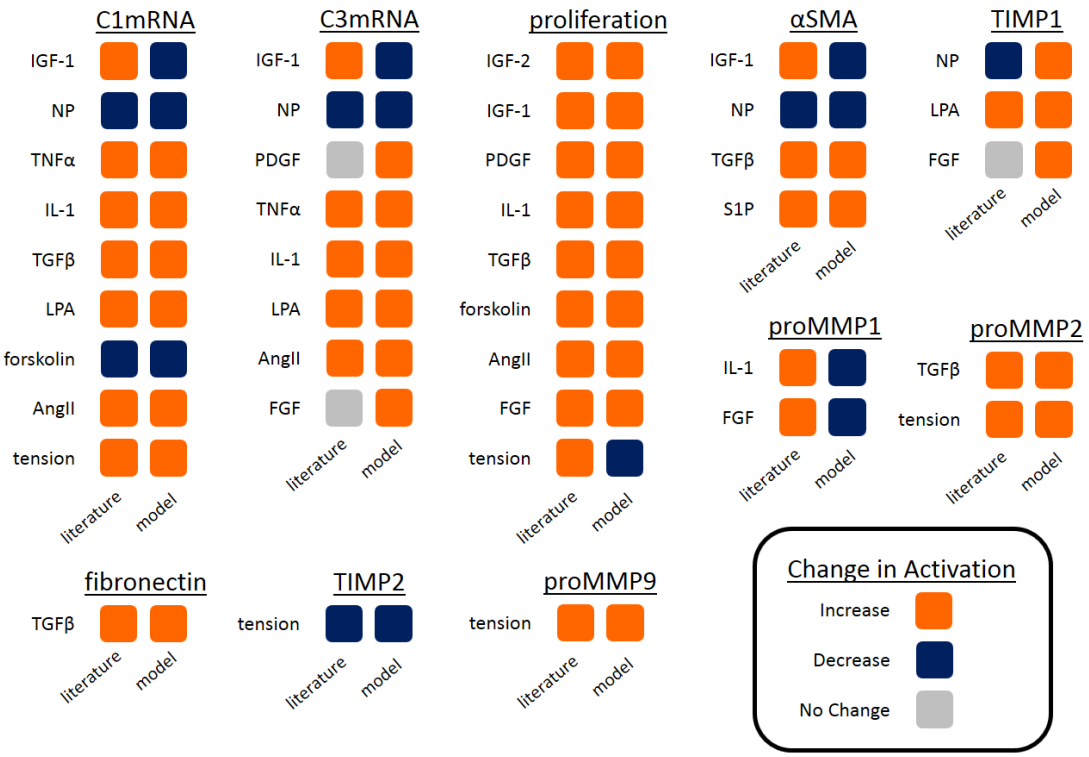

Figure 2: Model validation, comparison of model simulation predictions to literature data

Sensitivity Analysis

The all-inclusive results of both the blocking and positive treatment sensitivity analyses can be seen in Figures 3a and 4a. After performing the initial 111 sensitivity analysis simulations for both blocking and positive treatment, the change in activity data sets represented in Figures 3a and 4a were further analyzed to determine the most influential and the most sensitive species in the network model, shown in Figures 3b and 4b. The most influential nodes found from the blocking treatment sensitivity analysis included ROS, TGFβ, latent TGFβ, Rho, ROCK, AP1, S1P, SphK1, and S1P2. The most sensitive nodes found from the blocking treatment sensitivity analysis included NFAT, SRF, EDAFN, CImRNA, CIIImRNA, CI, CIII, cortactin, NWASP, and Arp23. The most influential nodes found from the positive treatment sensitivity analysis included AngII, AT1R, NOX, ROS, ET1, ETAR, TGFβ, latent TGFβ, PDGF, PDGFR, IL1, IL1RI, TNFα, TNFαR, TRAF, ASK1, JNK, abl, Rac1, MEKK1, MKK4, ERK, Raf, MEK1, FAK, AP1, FGF, and FGFR1. The most sensitive nodes found from the positive treatment sensitivity analysis included CREB, migration, proliferation, proMMP2, proMMP9, MMP2, MMP9, and MMP14.

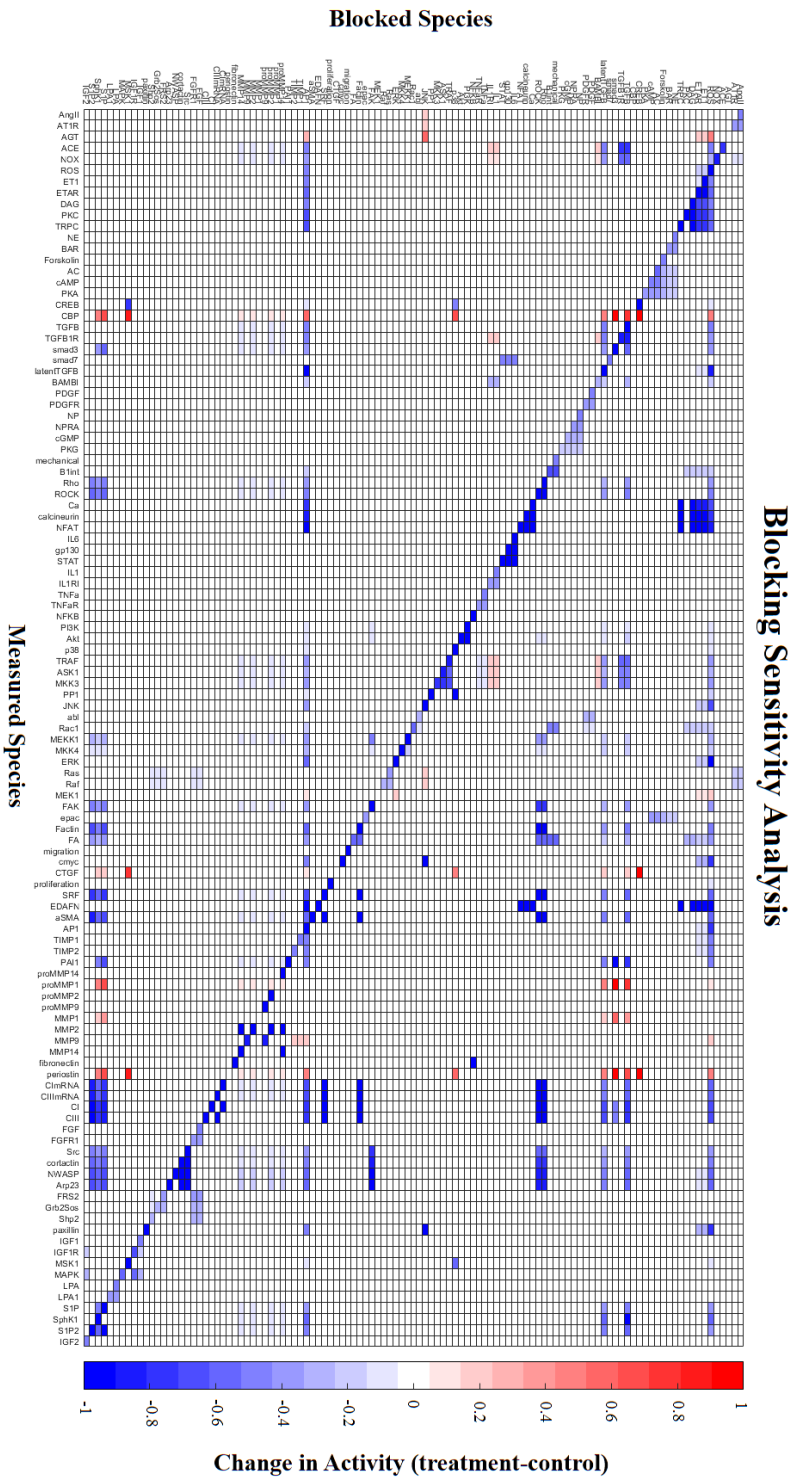

Figure 3a: Blocking treatment sensitivity analysis

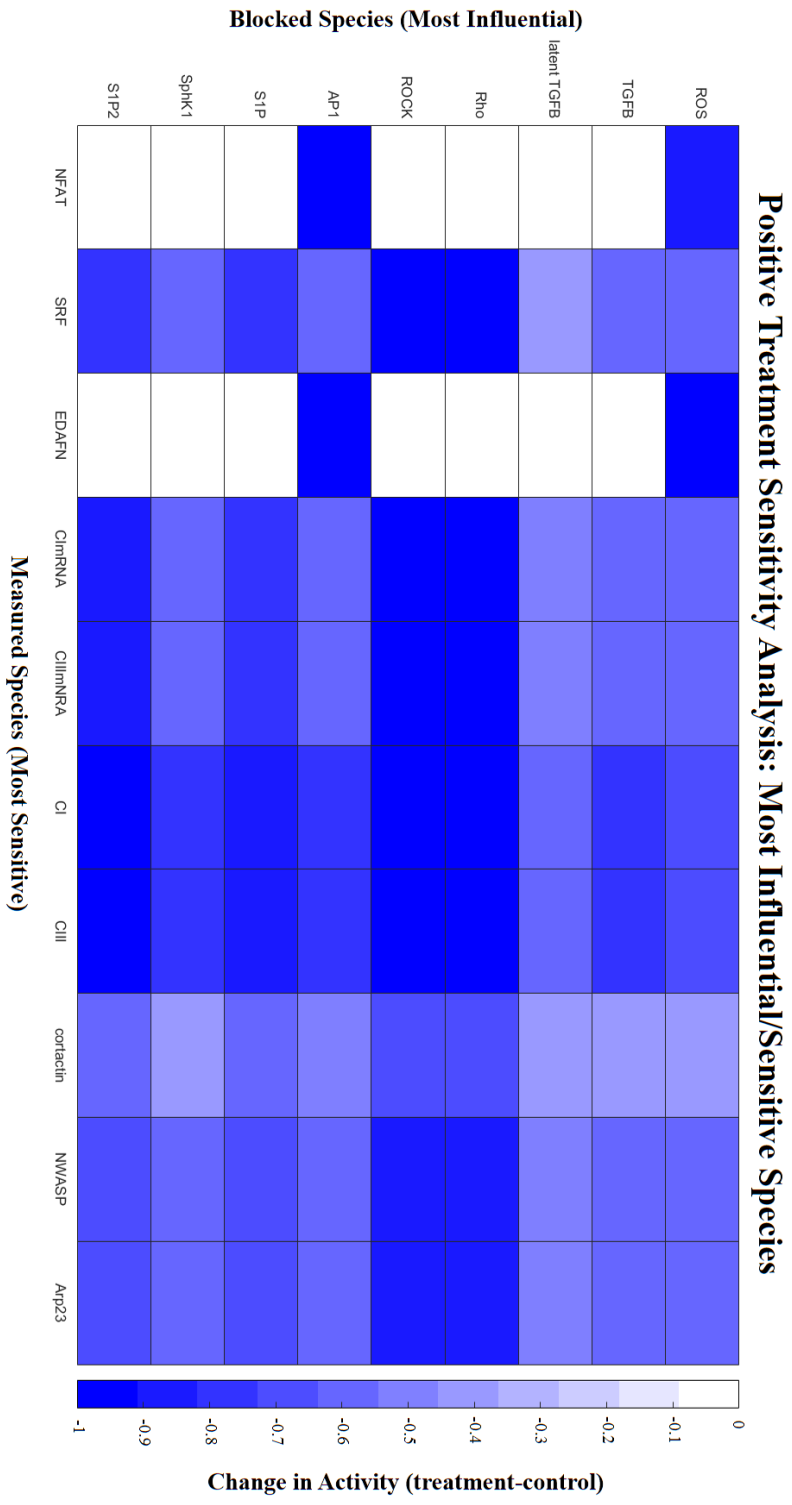

Figure 3b: Refined blocking treatment sensitivity analysis; the most influential and most sensitive species in the model

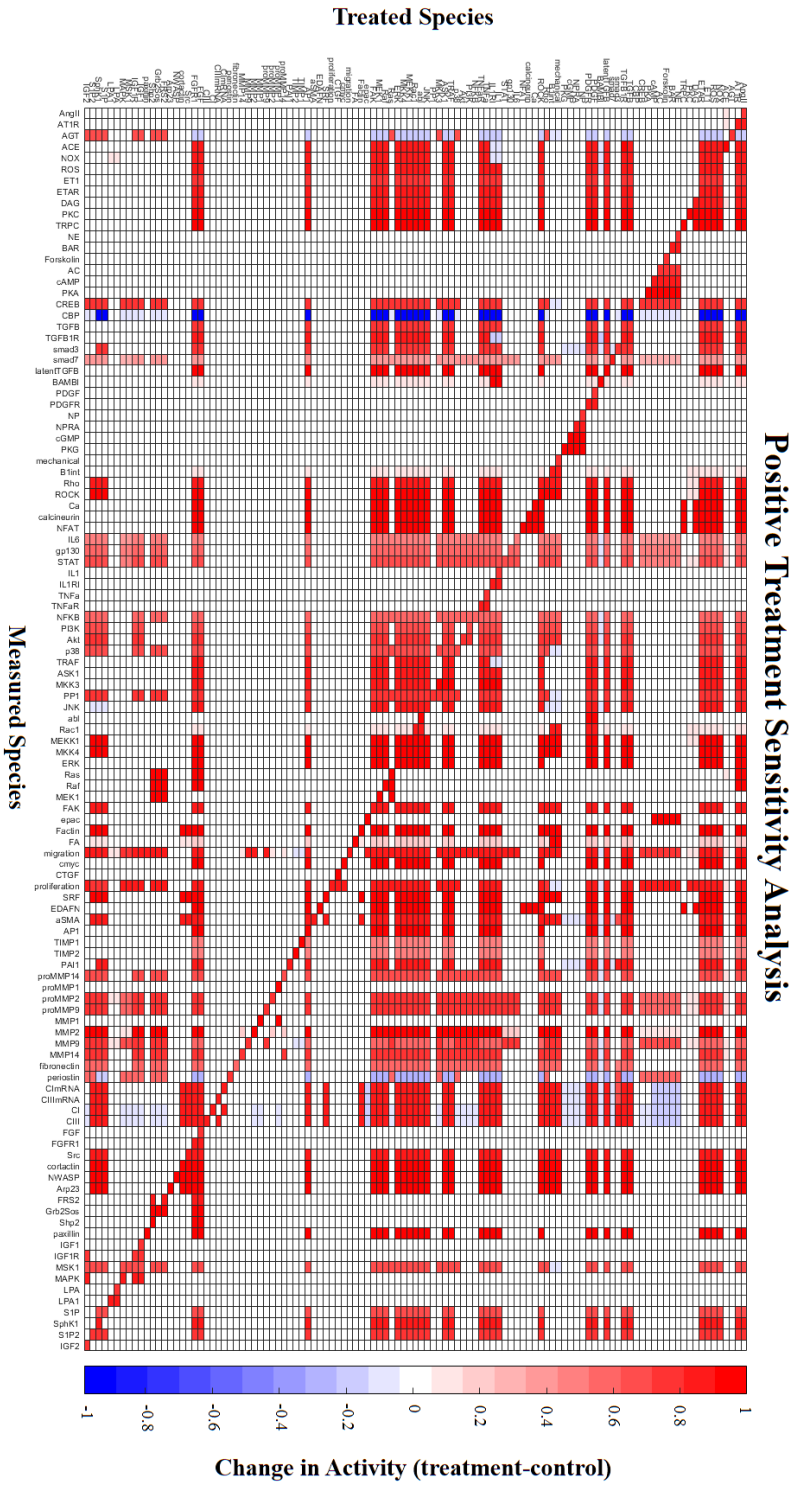

Figure 4a: Positive treatment sensitivity analysis

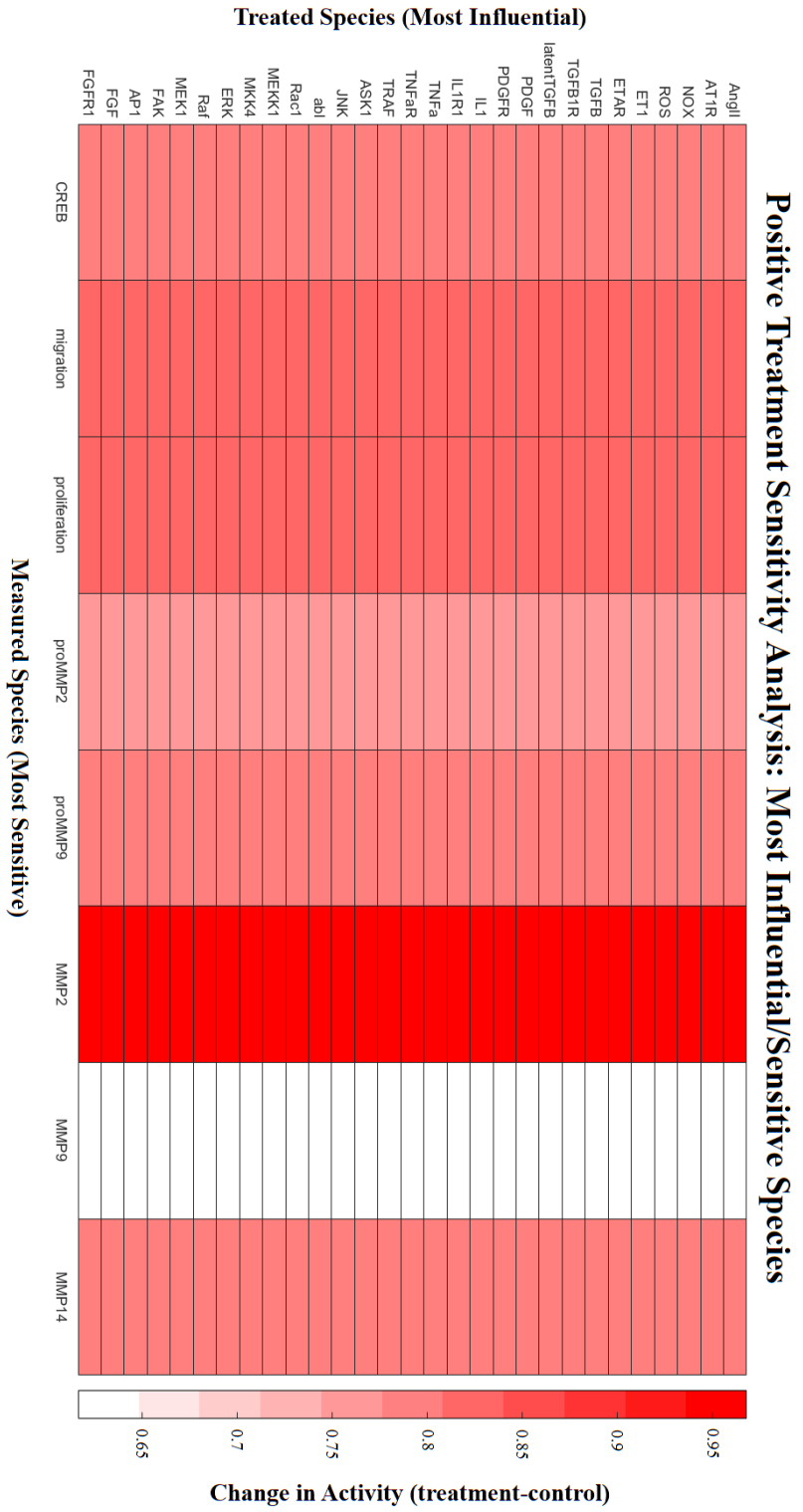

Figure 4b: Refined positive treatment sensitivity analysis; the most influential and most sensitive species in the model

MUSC IPF Drug Target Analysis

After performing both blocking treatment and positive treatment simulations, two sets of possible MUSC IPF drug targets were identified. The species most likely targeted by the MUSC IPF drug as identified by blocking treatment simulations included TGFβ, ROCK, Factin, and SRF. The species most likely targeted by the MUSC IPF drug as identified by positive treatment simulations included Shp2, FRS2, Ras, MKK3, p38, IGF1, IGF1R, and IGF2. The set of possible drug targets as identified by positive treatment analysis is larger due to the fact that, during analysis, all of the top 8 options listed above yielded equivalent simulation results. In other words, according to the model simulations all 8 are equally likely to be possible agonist targets. All predicted drug targets are identified in Figure 5.

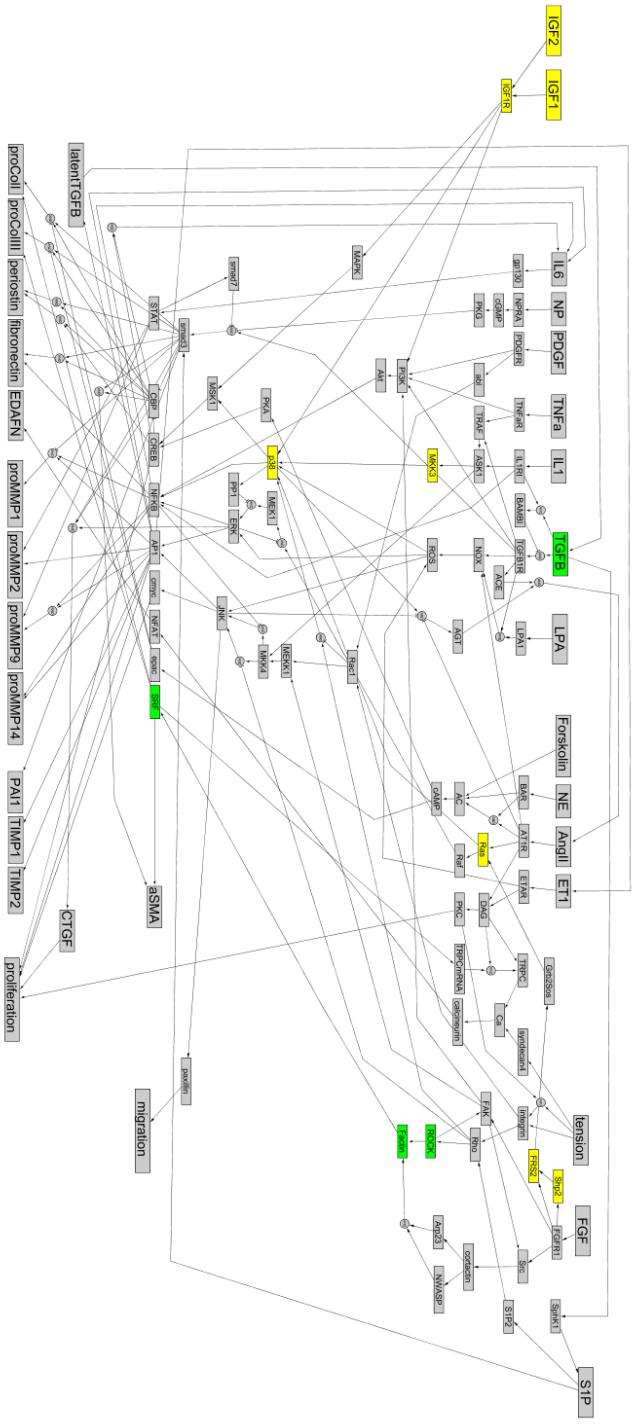

Figure 5: MUSC IPF drug target predictions; green boxes indicate targets identified by blocking treatment analysis; yellow boxes indicate targets identified by positive treatment analysis

CHAPTER FOUR

DISCUSSION

The complexity of idiopathic pulmonary fibrosis signaling is a significant factor for our incomplete understanding of its pathogenesis. Furthermore, this lack of understanding also adds to the difficulty experienced by researchers in developing effective treatments. Computational modeling is particularly useful in studying IPF more systemically and without wasting valuable resources in performing expensive and time-consuming physical experiments. Here we have constructed a computational model of fibrosis-related pulmonary fibroblast signaling network based on literature research. Network validation based on comparisons to pulmonary fibroblast literature data showed that the model has an accuracy of 74.4%. Multiple sensitivity analyses of the network were also performed in order to identify the most influential and the most sensitive species in the model.

Model Validation

Although the network model runs with an input-output prediction accuracy of 74.4%, multiple input-output relationships represented by literature data were predicted incorrectly. Some of the validation simulations that the model incorrectly predicted can be grouped together. The model incorrectly predicted all IGF1, all proMMP1, the majority of FGF, and the majority of TIMP1 validation simulations. These errors could be attributed to multiple things.

A first possibility is that our computational model has the ability to calculate very small responses that may not be distinguishable in a physical experiment or reproduced with a level of statistical significance. Note that our model predicted small responses in 3 cases in which literature reports found no significant change. Second, there is also the sure possibility that our model does not completely capture every molecule involved in the signaling pathways of interest. We specifically focused our model on previous reports of fibroblasts as a strategic choice to boost our confidence in the model's relevance to IPF. However, this choice brings the consequence that we have not included some molecular species that are thought to interact with our pathways but have not been demonstrated in fibroblasts. Third, there is of course the additional likelihood that other molecular species exist within this network but have not yet been discovered at all. It is noteworthy that our model itself can actually be used to help discover such unknown molecules by simulating the effect of mystery molecules and identifying what additional reactions in the model help fix our incorrect assumptions. Much like astronomers using celestial patterns to focus their searches for new stars, our model predictions identify locations in the network where we should be looking for new signaling species.

Sensitivity Analysis

Positive treatments induced mostly increases in activity levels, as can be seen by the majority of red coloring in Figure 4a indicating increases in activity level. Positive treatments also induced generally larger and more frequent changes in activity levels than

did blocking treatments. This could be due to challenges in determining baseline settings for different types of simulations. The baseline input reaction weights for positive treatment simulations were set at 0.25 and therefore had the possibility of deviating from baseline levels up to 0.75 compared to blocking simulations that only had the possibility of deviating from baseline levels a maximum of 0.5. There are also more activating reactions in the model than inactivating reactions which could also lead to positive treatment yielding overall greater changes in activity levels. In contrast to positive treatments, blocking treatments induced mostly decreases in activity levels, as can be seen by the majority of blue coloring in Figure 3a indicating decreases in activity level.

The majority of the most influential and most sensitive species identified in both the blocking and positive treatment sensitivity analysis do not overlap. However, in both treatment scenarios both TGFβ (transforming growth factor β) and AP1 (activator protein 1) were identified as some of the most influential species in the model. After performing thorough literature research it's clear that TGFβ plays a central role in the innerworkings of fibroblasts, including pulmonary fibroblasts. Thus, it's not surprising that the sensitivity analysis simulations show that TGFβ plays a central role in the constructed network model as well. AP1 has a subunit, Jun, that was also repeatedly indicated as an important signaling molecule in the activities of pulmonary fibroblasts during model construction literature research. Therefore, like TGFβ, it's not surprising that the sensitivity analysis has also identified AP1 as playing a central role in the model. However, there is also a possibility that TGFβ and AP1 have been identified as centrally important signaling molecules in the model due to a bias that may come from the

literature. While it may be true that these species do indeed play significant roles physiologically, there is also a possibility that these molecules were identified early in studying fibroblasts and thus there is now an over-saturation of literature regarding their roles in fibroblast signaling. It must then be recognized that other signaling molecules in the model have the possibility of playing larger roles physiologically than the model may now suggest due to a lack of literature information regarding their roles in fibroblast activity.

MUSC IPF Drug Target Analysis

Although 12 total possible drug targets were identified by initial model simulations, these can be further analyzed and the resulting drug target predictions refined. After observing where the identified species are located within the signaling network it can be seen that multiple initial identified species are directly upstream or downstream of other initially identified species and can thus be grouped together by effect (e.g., IGF-1 and IGF1R, MKK3 and p38, ROCK and F-actin). While the model is not able to predict any differences between perturbing these "linear" linkages in the network, experiments can of course be designed to identify whether the experimental drug binds to the more-upstream or more-downstream species.

Future

In the specific context of the preliminary MUSC IPF drug, laboratory experiments can now directly test the identified possible drug target interactions. The refined drug target predictions (TGFβ, ROCK, SRF, MKK3, Ras, Shp2, IGF1, and IGF2) are the recommended species with which to begin experimental investigations. Similar to how the model has been used to advance the understanding of the MUSC IPF drug, this model can be used to do the same with other lung associated pharmaceuticals. This model can also be applied to further identify other desirable drug targets for treating IPF as well as other lung diseases that affect pulmonary fibroblasts.

Also in the context of drug therapy, the model can be applied in the study of combinatory therapies for various lung diseases, allowing researchers to identify drugs that may have a synergistic effect when used in combination with one another. A final suggested application for this model is in the context of personalized medicine, including both prognosis and therapy selection. For example, the model has the ability to be adjusted in order to be more representative of one genome, thus allowing researchers to investigate the best possible treatment options for that individual patient.

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