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EFFECT OF NEGATIVE FEEDBACK LOOP WITH NRF1 AND MIR-378 OF NONALCOHOLIC FATTY LIVER DISEASE: A MATHEMATICAL MODELING APPROACH

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ABSTRACT. Nonalcoholic fatty liver is a type of fatty liver in which fat accumulates in the liver without alcohol. In the accumulation, Nrf1 and miR-378 genes play very important role, so called negative feedback loop, in which the two genes suppress the other's production. In other words, Nrf1 activates fatty acid oxidation which promotes fat consumption in the liver, while miR-378 deactivates fatty acid oxidation. Thus, both genes regulate nonalcoholic fatty liver.

In this paper, the negative feedback loop of Nrf1 and miR-378 are expressed by a system of ordinary differential equations. And, bifurcation simulation shows the change in the amount of each gene with significant parameter range changes. Bifurcation simulation has also used to determine the thresholds for transit between disease and steady state.

1. Introduction

Nonalcoholic fatty liver disease(NAFLD) is a disease in which liver triglyceride accumulates regardless of alcohol consumption. NAFLD includes various diseases of liver pathologies ranging from simple steatosis to inflammation of varying degrees, hepatocyte injury and fibrosis [3]. Without intervention, it can progress to end-stage liver disease and hepatocellular cancer. Risk of hepatocellular cancer is, in fact, significantly higher in NAFLD patients than in the general clinical population [12].

A number of studies have proposed mathematical models that focus on the genetic factors in various severe diseases, including cancer [13, 14, 15]. These studies establish their mathematical models using experimental data. The advantage of the modelling is that they can provide predictions and potential

insights beyond limited experimental data, generate new hypotheses, and suggest disease treatment strategies for future testing. Thus, in this study, NAFLD caused by genetic factors is approached using mathematical models.

Recent researches on the pathogenesis of NAFLD have considered small non-coding RNAs, called microRNAs(miRNAs). They have been shown to be important regulators of lipids [8]. In particular, miR-378 is an upregulated target of nuclear respiratory factor 1(Nrf1) in the liver [7]. The relation between Nrf1 and miR-378 is as follows; Nrf1 plays a role in mediating the activation of the fatty acid oxidation(FAO) process [10] and the expression of miR-378 is negatively regulated by Nrf1 and acts as a transcriptional inhibitor that forms a negative feedback loop in which there is overexpression of miR-378-impaired FAO, which is regulated via the suppression of Nrf1. Hence, a negative feedback loop between miR-378 and Nrf1 regulates the NAFLD. According to the experimental paper, the reduction of miR-378 is assumed to be a potential treatment for NAFLD.
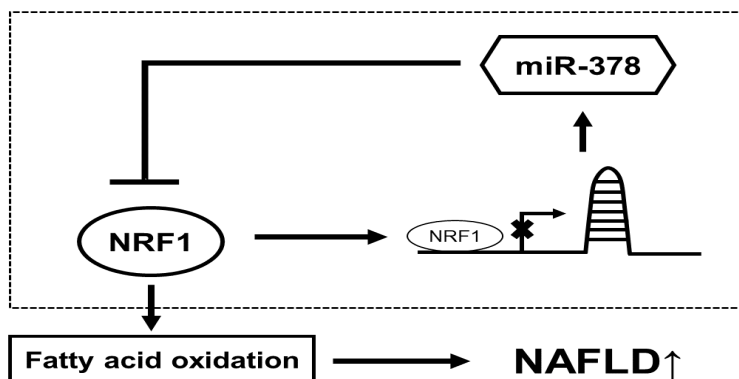

FIGURE 1. Negative feedback loop circuit between miR-378 and Nrf1[7]

Since this negative feedback loop can be established a mathematical model for NAFLD, we would like to confirm the hypothesis about the possibility of NAFLD treatment by regulating the amount of miR-378. Even though the relevant experimental data have not yet collected, in our knowledge, on this hypothesis, a mathematical model provides the possibility of confirming or refuting on the hypothesis.

2. Gene regulatory network

All cellular functions are driven by proteins. Protein production occurs through a gene expression process that involves the reading of information encoded in DNA. The cellular abundance of each protein is controlled primarily by its production rate which is in turn controlled by specialized proteins called transcription factors. A set of genes whose protein products regulate one another expression rates is referred to as a gene regulatory network. In this section, we will address gene regulatory networks that implement switch-like responses. Gene expression is a two-step process. The first step, which is called transcription, occurs when the coding region of a gene is rewritten in the form of a complementary RNA strand called a messenger RNA(mRNA). Transcription is carried out by a protein complex called RNA polymerase that binds the promoter region of the gene and then walks along the DNA, catalyzing the formation of the mRNA strand from nucleotide precursors. The second step of gene expression is translation, in which the mRNA molecule binds a protein RNA complex called a ribosome that reads the nucleotide sequence and produces a corresponding polypeptide chain. Translation, like transcription, involves information transfer that the ribosome reads along the mRNA and catalyzes the formation of a protein from amino acids building blocks. We will use the law of mass action and a formalism on the basis of the Hill equation to develop models of gene regulatory networks [6].

Definition 1. (Law of mass action) The law of mass action states that the rate of any given chemical reaction is proportional to the product of concentrations of the reactants.

For more specific explanation, we consider the case in which reactants R_1 and R_2 react together to give products P_1 and P_2,

$$aR_1 + bR_2 \rightleftharpoons cP_1 + dP_2,$$
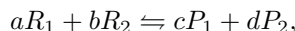
where a, b, c and d are the number of moles of the corresponding reactants and products. Then, according to the law of mass action,

$$\text{Rate of forward reaction} \propto [R_1]^a[R_2]^b$$
$$\implies \text{Rate of forward reaction} = K_f[R_1]^a[R_2]^b,$$
$$\text{Rate of backward reaction} \propto [P_1]^c[P_2]^d$$
$$\implies \text{Rate of backward reaction} = K_b[P_1]^c[R_2]^d,$$

where K_f and K_b are the rate constants for forward and backward reaction, respectively. At equilibrium state, the rate of forward reaction becomes equal to the rate of backward reaction. i.e.,

$$K_f[R_1]^a[R_2]^b = K_b[P_1]^c[R_2]^d \quad \text{or} \quad \frac{K_f}{K_b} = \frac{[P_1]^c[P_2]^d}{[R_1]^a[R_2]^b}.$$

Therefore, $K = K_f/K_b$ is called as the *equilibrium constant* or more specifically the *dissociation constant*. Dissociation constant measure the tendency of a

larger object to fall apart into its separate subunits or components. Its value is determined by experimental data and gives an indication on the degree to which dissociation occurs. If the dissociation constant is small, then there is a high affinity between the components [9].

Definition 2. (Hill equation) The Hill equation, which was derived from the Michaelis-Menten kinetics, describes the enzyme reaction mechanism based on the law of mass-action. The general Hill equation form is

$$nL + R \leftrightharpoons L_n R, \qquad [L_n] = [R_0]\frac{[L]^n}{[L]^n + K_d} = [R_0]\frac{[L]^n}{[L]^n + (K_A)^n}$$

where L is the ligand that can be present in variable concentration; R is the receptor, amount of which is constant and is significantly exceeded by the amount of the ligand; $[L_n R]$ is the concentration of the ligand-receptor complex; $[R_0]$ is the total receptor concentration(receptor number); $[L]$ is the concentration of the free ligand; k_1 and k_2 are the rate constants of association and dissociation, respectively; $K_d = k_2/k_1$ is the equilibrium dissociation constant of the ligand-receptor complex; K_A is the ligand concentration, at which half the receptors are ligand-bound(if $n = 1$, it equals the K_d); n is originally, the number of binding sites for the given ligand in one receptor [11].

We note that we obtain a model which is modeled by Michaelis-Menten kinetics when $n = 1$.

We introduce the modelling process of gene input functions using Hill equation. First, we consider the following reaction to focus on the binding of repressor;

$$X + D \xrightleftharpoons[K_b]{K_f} [XD].$$
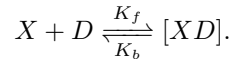
Here, in the forward reaction, the transcription factor protein, X binds to the binding site, D of the promoter to form the complex XD at the rate K_f. In the backward reaction XD is dissociating into X and D at the rate K_b. Transcription of the gene occurs only when X is not bound or when D is free. In fact, most transcription factors are composed of multiple subunits and in order to achieve maximum activity these multiple subunits cooperatively bind the binding site.

Next, we suppose there are n subunits. Then the Hill equation of input function of the gene bound with repressor is

$$\text{Promoter activity} = \frac{\beta}{1 + ([X]/K_d)^n}$$

and n is called as the *Hill coefficient* [2]. Promoter activity is defined as the number of RNA polymerase molecules that pass by the final base pair of the promoter and continue along the DNA as an elongation complex. In general, measuring gene products like mRNA and protein has been the approach used to measure promoter activity [18].

3. Mathematical Modeling

3.1. Double-negative feedback loop model

Negative feedback occurs when a cause produces an effect and the result suppresses the cause. In biology, it appears in the regulation of hormone levels, body temperature control, and reactions between various enzymes. Double-negative feedback loops mean that the two elements eventually suppress each other. In [17], miR-378 and Nrf1 produce a double-negative feedback loop because they constrain each others output. In general, double-negative feedback loops have included a 'transition' that controls results and can be represented through a mathematical model [17].

3.2. NAFLD model description

The negative feedback loop between miR-378 and Nrf1 controlling NAFLD is briefly depicted in Figure 2. As a result of the experiments previously mentioned, miR-378 upregulates NAFLD by decreasing FAO through the inhibition of Nrf1 expression. Meanwhile, Nrf1 downregulates NAFLD by increasing FAO through its inhibition of miR-378 expression.

N	Concentration of Nrf1
m	Concentration of miR-378
k_1	Maximal transcription rate of Nrf1
k_2	Michaelis constant for Nrf1 production catalase by miR-378
γ	Degradation rate of Nrf1
k_3	Maximal transcription rate of miR-378
k_4	Michaelis constant for miR-378 production catalase by Nrf1
δ	Degradation rate of miR-378

TABLE 1. Variables and parameters used in NAFLD model.

Here, we focus on the model of the feedback loop between miR-378 and Nrf1, which controls the phenotype with NAFLD or the normal phenotype :

$$\frac{dN}{dt} = \frac{k_1}{1 + k_2 m} - \gamma N \tag{1}$$

$$\frac{dm}{dt} = \frac{k_3}{1 + k_4 N^2} - \delta m \tag{2}$$

The scheme of the NAFLD model is illustrated in Figure 2. The first term of (1) represents the promoter activity of Nrf1 under the inhibition of miR-378. Similarly, the first term of (2) reveals the promoter activity of miR-378 under the inhibition of Nrf1. Both of the first terms consider the reaction :

$$N + m \underset{f}{\overset{e}{\rightleftharpoons}} [Nm], \tag{3}$$

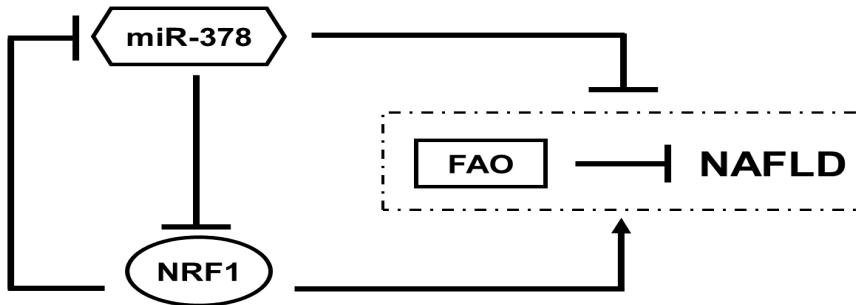$$m + N \underset{h}{\overset{g}{\rightleftharpoons}} [mN]. \tag{4}$$

FIGURE 2. Schematic diagram of negative feedback loop between miR-378 and Nrf1

$[Nm]$ and $[mN]$ are not equal since, even Nrf1 and miR-378 inhibit and regulate each other, the react to inhibit sites are different. Therefore, according to reactions (3) and (4), the promoter activities of Nrf1 and miR are

$$\frac{k_1}{1+\dfrac{e}{f}m} = \frac{k_1}{1+k_2m} \quad \text{and} \quad \frac{k_3}{1+\dfrac{g}{h}N^2} = \frac{k_3}{1+k_4N^2},$$

respectively. The rate of synthesis of Nrf1 is indicated by the first term in the equation (1). k_1 represents maximal constitutive protein expression, where the denominator $(1+k_2m)$ indicates miR-378 dependent downregulation of Nrf1 expression. Similarly, the repression of Nrf1 by miR-378 is performed equation (2). The rate of synthesis of miR-378 repressed by Nrf1 represents the first term. k_3 is a parameter that describes the maximal miR-378 production rate. The denominator of that term performed Nrf1 dependent downregulation of miR-378 expression, where Hill coefficient 2 can arise binding of Nrf1 to two sites in the region of miR-378.

4. Mathematical Analysis

In this section, the qualitative behavior of the NAFLD model is discussed. In particular, we investigated the existence of the positive equilibrium point of the model and its local asymptotic stability. Moreover, the bifurcation diagrams demonstrated qualitative changes. First, however, we explored whether the model implied biological significance, equilibrium solutions, and stability analysis.

4.1. Equilibrium solutions and stability analysis

Since the NAFLD model describes biological phenomena, it is very important to prove that all of the states, variables, and parameters are nonnegative with respect to time. In other words, we had to prove that solution of the model with positive initial values remains positive at all times $t \geq 0$.

Theorem 4.1. *The region $D = \{(N,m) \,|\, N \geq 0, m \geq 0, \frac{k_1}{1+k_2 m} \geq 0, \frac{k_3}{1+k_4 N^2} \geq 0\}$ is positively invariant for the NAFLD model.*

Proof. The state variables (N, m) that remain in the biologically meaningful region $\{(N,m) \mid N \geq 0, m \geq 0\}$ is positively invariant for NAFLD model. In addition, all of the model parameters are supposed to be strictly positive constants. We calculated the model as follows ;

$$\left.\frac{dN}{dt}\right|_{N=0} = \frac{k_1}{1 + k_2 m} \geq 0, \qquad \left.\frac{dm}{dt}\right|_{m=0} = \frac{k_3}{1 + k_4 N^2} \geq 0. \tag{5}$$

Equations in (5) indicate that $\dfrac{dN}{dt}$ and $\dfrac{dm}{dt}$ are always non-negative at all times $t > 0$. Therefore, the positive invariant for the model region is D, which is biologically valid. □

Theorem 4.2. *Let the system (1)–(2) has the NAFLD-free equilibrium $E_0 = (N_0, 0)$. Then the E_0 is locally asymptotically stable if $C_1 > 0$ and it is unstable if $C_1 < 0$ where $C_1 = \gamma\delta - \dfrac{2k_1 k_2 k_3 k_4}{\sqrt{1 + k_4 N_0^2}\sqrt{1 + k_2}}$.*

Proof. Suppose $E_0 = (N_0, 0)$ is equilibrium point. Then we get

$$0 = k_1 - \gamma N_0 \tag{6}$$

$$0 = \frac{k_3}{1 + k_4 N_0^2} \tag{7}$$

Taking $m = 0$ in (2), we have $k_1 - \gamma N = 0$. Since the equilibrium of our system (2) leads to $N_0 = \dfrac{k_1}{\gamma}$, we get $E_0 = (\dfrac{k_1}{\gamma}, 0)$. Before computing the Jacobian matrix at E_0, it is useful to choose a suitable order for the components, N and m. The Jacobian matrix evaluated at E_0 is the form

$$J(E_0) = \begin{pmatrix} -\gamma & \dfrac{-k_1 k_2}{\sqrt{1 + k_2}} \\ \dfrac{-2k_3 k_4 N_0}{\sqrt{1 + k_4 N_0^2}} & -\delta \end{pmatrix}$$

The characteristic equation of E_0 is

$$f(\lambda) = \lambda^2 + (\gamma + \delta)\lambda + \gamma\delta - \frac{2k_1 k_2 k_3 k_4}{\sqrt{1 + k_4 N_0^2}\sqrt{1 + k_2}} = \lambda^2 + (\gamma + \delta)\lambda + C_1 = 0$$

Hence, we get $\text{Tr}(J) = -(\gamma + \delta)$ and $\det(J) = C_1$. Since every parameters are non-negative, clearly $\text{Tr}(J) < 0$. By the Routh-Hurwitz criterion [1], $E_0 = (N_0, 0)$ is locally asymptotically stable if $C_1 > 0$ and it is unstable if $C_1 < 0$. □

Biologically miR-378-ASO(anti-sense oligonucleotide) treatment significantly reduces miR-378 in hepatocytes[7]. We assumed this state as E_0. In conclusion, this local asymptotic stability says that solutions having initial values near E_0 move to the point E_0 eventually. This fact indicates that if m is not present, it converges to E_0.

Theorem 4.3. *Let the system (2)–(3) has infected NAFLD equilibrium $E_1 = (N_1, m_1)$ with $m_1 > 0$. Then E_1 is locally asymptotically stable if $C_2 > 0$ and it is unstable if $C_2 < 0$ where $C_2 = \gamma\delta - \dfrac{2k_1 k_2 k_3 k_4 N_1}{\sqrt{1 + k_4 N_1^2}\sqrt{1 + k_2 m_1}}$.*

Proof. Suppose $E_1 = (N_1, m_1)$ is equilibrium point. Then we get

$$0 = \frac{k_1}{1 + k_2 m_1} - \gamma N_1^2, \tag{8}$$

$$0 = \frac{k_3}{1 + k_4 N_1^2} - \delta m_1. \tag{9}$$

we have $m_1 = \dfrac{k_3}{\delta(1 + k_4 N_1^2)}$. Substituting (9) into the first equation of (8), we obtain

$$\gamma\delta k_4 N_1^3 - \delta k_1 k_4 N_1^2 + (\gamma\delta + \gamma k_2 k_3)N_1 - \delta k_1 = 0 \tag{10}$$

The cubic equation of N_1 may have up three solutions. There are two complex solutions and one non-negative solutions such as

$$N_a = \frac{k_1}{3\gamma} - \frac{A}{3\gamma\delta k_4} - \frac{B}{3\gamma\delta k_4},$$

$$N_b = \frac{k_1}{3\gamma} + \frac{(1 + i\sqrt{3})A}{6\gamma\delta k_4} + \frac{(1 - i\sqrt{3})B}{6\gamma\delta k_4},$$

$$N_c = \frac{k_1}{3\gamma} + \frac{(1 - i\sqrt{3})A}{6\gamma\delta k_4} + \frac{(1 + i\sqrt{3})B}{6\gamma\delta k_4}.$$

Here, $A^3 = \dfrac{C_3 + \sqrt{C_4}}{2}$ and $B^3 = \dfrac{C_3 - \sqrt{C_4}}{2}$ where

$$C_3 = -2\delta^3 k_1^3 k_4^3 + 9\gamma\delta^2 k_1 k_4^2(\gamma\delta + \gamma k_2 k_3) - 27\gamma^2\delta^3 k_1 k_4^2,$$
$$C_4 = C_3 - 4(\delta^2 k_1^2 k_4^2 - 3\gamma\delta k_4(\gamma\delta + \gamma k_2 k_3))^3.$$

Since only the non-negative solution is biologically relevant, we can choose $N_1 = N_a = \dfrac{k_1}{3\gamma} - \dfrac{A}{3\gamma\delta k_4} - \dfrac{B}{3\gamma\delta k_4}$. Putting N_a in (6), $m_1 = \dfrac{2\delta k_1 k_4 + A + B}{k_2(\delta k_1 k_4 - A - B)}$. Therefore, we get

$$E_1 = (N_1, m_1) = (\frac{\delta k_1 k4 - A - B}{3\gamma\delta k_4}, \frac{2\delta k_1 k_4 + A + B}{k_2(\delta k_1 k_4 - A - B)}).$$

If $\delta k_1 k4 - A - B > 0$, then E_1 is always a non-negative equilibrium point. Computing the Jacobian matrix at E^* for (6), we have

$$J(E^*) = \begin{pmatrix} -\gamma & \dfrac{-k_1 k_2}{\sqrt{1 + k_2 m_1}} \\ \dfrac{-2k_3 k_4 N_1}{\sqrt{1 + k_4 N_1^2}} & -\delta \end{pmatrix}$$

The characteristic polynomial of E_1 is

$$f(\lambda) = \lambda^2 + (\gamma + \delta)\lambda + \gamma\delta - \frac{2k_1 k_2 k_3 k_4 N_1}{\sqrt{1 + k_4 N_1^2}\sqrt{1 + k_2 m_1}} = \lambda^2 - Tr(J)\lambda + det(J).$$

Since every parameter is positive, clearly $Tr(J) < 0$. Therefore, if $C_2 > 0$ then E_1 is locally asymptotically stable and it is unstable if $C_2 < 0$ where

$$C_2 = \gamma\delta - \frac{2k_1 k_2 k_3 k_4 N_1}{\sqrt{1 + k_4 N_1^2}\sqrt{1 + k_2 m_1}}.$$

□

4.2. Numerical results

In this section, we simulated the effect of the Nrf1 and miR-378 negative feedback loop on NAFLD. Figure 3 and Figure 4 show the bifurcation diagrams of Nrf1 and miR-378 with the change of range $k3$. Figure 5 and Figure 6 show the bifurcation diagrams of Nrf1 and miR-378 with the change of range $k1$ in Figure 4.

In Figure 3 and Figure 4, when k_3 is small, the Nrf1 level is higher than the miR-378 level. Thus, the phenotype appears in a normal state. In contrast, when k_3 is large, the miR-378 level is higher than Nrf1. In the case, the opposite reaction occurs; that is, the NAFLD phenotype appears. The regions with an intermediate k_3 represent two positive stable equilibria (depicted by solid line). In general, that is called a bistability region. Between the two stable equilibria, there is an unstable equilibrium (depicted by a dashed line). When k_3 becomes large, the state of the region of the phenotype changes from its normal state to NAFLD. Conversely, when k_3 becomes small, the opposite change occurs. As k_3 gradually increases or decreases, the point at which the phenotype begins to change is BP and is called the threshold.

Similarly, in Figure 5 and Figure 6, when k_1 is small, the Nrf1 level is smaller than the miR-378 level. Thus, the phenotype represents NAFLD state. In contrast, when k_1 is large, the Nrf1 level is larger than the miR-378 level. In this case, the opposite reaction occurs, that is, the normal phenotype appears. Figure 5 and Figure 6 similar to Figure 3 and Figure 4, the regions with an intermediate k_1 represent two positive stable equilibria (depicted by a solid line). In general, it is called a bistability region. Between the two stable equilibria represent unstable equilibrium (depicted by a dashed line). When k_1 becomes large, the state of the region of the phenotype changes from NAFLD to normal
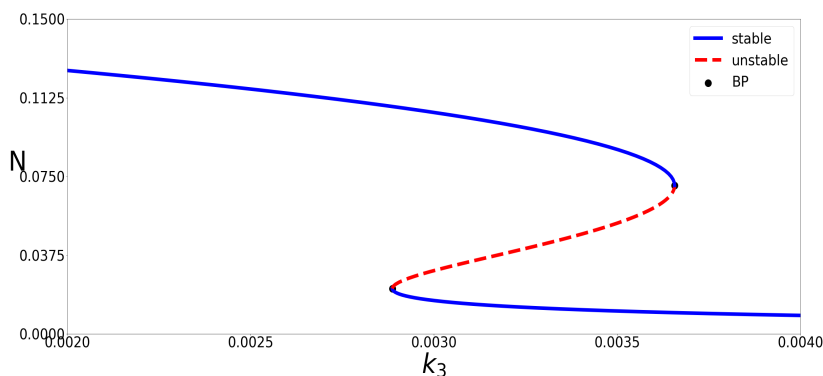
FIGURE 3. Bifurcation diagram of N with the change of k_3. The Nrf1 and miR-378 levels at equilibria. The parameter values are: $k_1 = 0.05$, $k_2 = 100$, $k_4 = 3000$, $\gamma = 0.33$, $\delta = 0.02$.
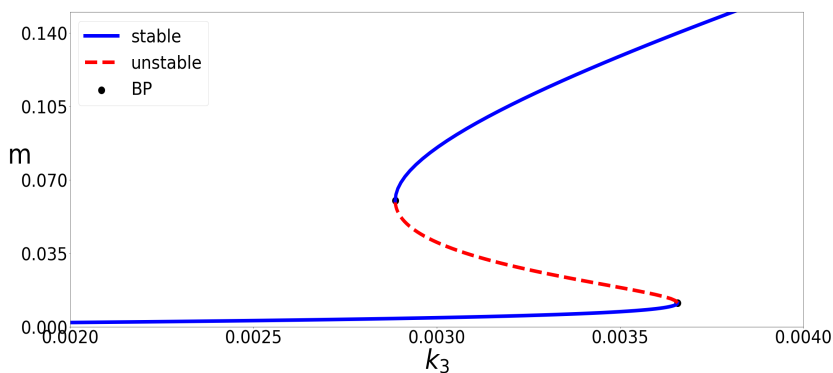

FIGURE 4. Bifurcation diagram of m with the change of k_3. The Nrf1 and miR-378 levels at equilibria. The parameter values are: $k_1 = 0.05$, $k_2 = 100$, $k_4 = 3000$, $\gamma = 0.33$, $\delta = 0.02$.

state. Conversely, when k_1 becomes small, the opposite situation occurs. As k_1 gradually increases or decreases, the point at which the phenotype begins to change is BP and is called the threshold. Overall, therefore, the disease phenotype is changed when k_3 is exceeded by certain thresholds of Nrf1 and miR-378. It is the fact that maximal transcription rate of miR-378 is the key of NAFLD therapy.
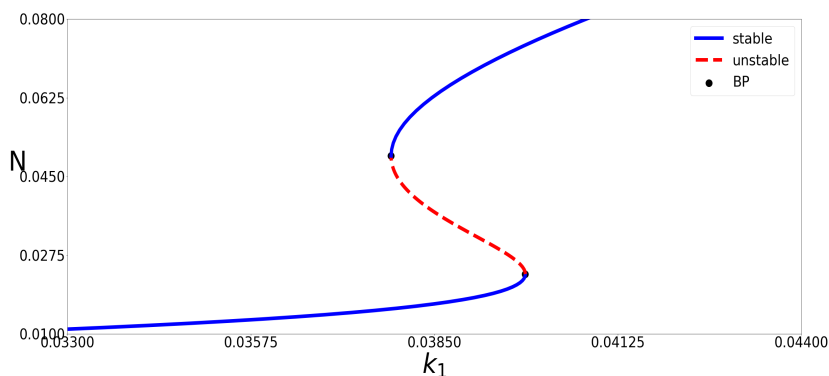
FIGURE 5. Bifurcation diagram of N with the change of k_1. The Nrf1 and miR-378 levels at equilibria. The parameter values are: $k_2 = 100$, $k_3 = 0.0022$, $k_4 = 3000$, $\gamma = 0.33$, $\delta = 0.02$.

FIGURE 6. Bifurcation diagram of m with the change of k_1. The Nrf1 and miR-378 levels at equilibria. The parameter values are: $k_2 = 100$, $k_3 = 0.0022$, $k_4 = 3000$, $\gamma = 0.33$, $\delta = 0.02$.
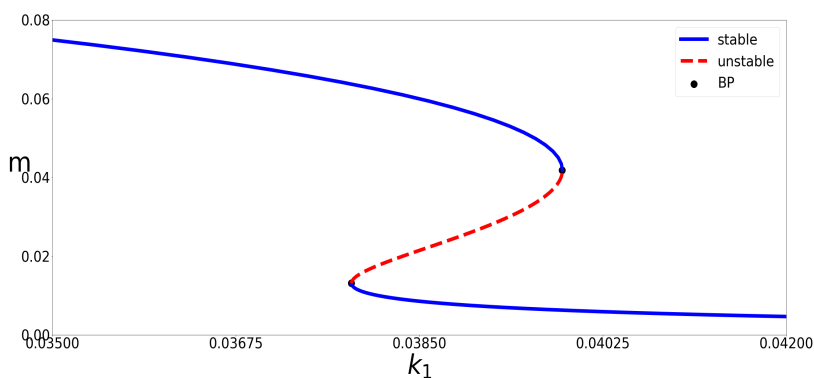
5. Discussion

In a previous study, the therapeutic potential of miR-378 reducing drugs is mentioned [7]. In this study, a nonlinear mathematical model for NAFLD was established for two genes, miR-378 and Nrf1. The mathematical model yielded two asymptotical stable intervals of NAFLD-free equilibria and one NAFLD equilibria. We obtained bifurcation to observe the change of Nrf1 and miR-378 concentrations depending on the range of $k1$ and $k3$. Further, analysis of the model revealed the presence of a transition in NAFLD. Analysis

of the model revealed the presence of a transition in NAFLD; an "on transition" from the normal state to the NAFLD state and an "off transition" from the NAFLD state to the normal state. This mathematical modeling study shows that the treatment of the disease can be enhanced by switching off NAFLD by the regulation of miR-378.

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