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Controlling the abnormal state in the Rb-E2F pathway involving microRNAs

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Experiments have shown that microRNAs play an important role in regulating gene networks. They can orchestrate whole cellular programs by simultaneously regulating the expression of a group of genes. Restoring microRNAs to normal levels in cancer cells can reverse the aberrant cell growth. So understanding how to control the normal levels of microRNAs in the cancer cells is a critical step in controlling cancer. In this paper, a method to control microRNAs levels is presented by investigating the gene regulative network involving the Rb-E2F pathway and microRNAs (miR449); also, the influence of noises on the dynamical behaviour of the Rb-E2F pathway is studied by using a mathematical model. The results show that there is a relationship between noise density and system response. Through finding and defining the phenomenon of stochastic resonance it was found that the stochastic resonance can be controlled through the optimal noise intensity, and the microRNA levels can be controlled. These findings are propitious in providing a novel way to heal cancer.

Keywords: MicroRNAs, Noise, Stochastic resonance.

INTRODUCTION

Maintenance of normal cell function and tissue homeostasis is dependent on the precise regulation of multiple signaling pathways [1]. Once the balance is destroyed, genetic diseases such as cancer arise. Rb and E2F proteins play important roles in the regulation of cell division, cell growth, and programmed cell death by controlling the expression of genes involved in these processes; they are best known for their regulation of the cell cycle at the G1/S transition [2]. The Rb gene was the first identified tumor suppressor gene [3], and it now is recognized as playing a fundamental role in a signaling pathway that controls cell proliferation [1]. Rb regulates the transcription of genes that are essential for DNA replication and cell cycle progression by binding and inhibiting E2F transcription factors [4]. In recent years, a large number of studies have focused on the mechanisms controlling cellular proliferation associated with human cancer experimentally regulated by the Rb-E2F pathway [5-8]. As we all know, microRNAs (miRNAs) are an abundant class of tiny RNAs that regulate the expression of protein-coding genes in plants and animals [9]. They are thought to have the capacity for serving as diagnostic and prognostic biomarkers in human cancers [10]. This paper mainly focuses on miR-449, which can induce cell senescence and apoptosis and act as a tumor suppressor through regulating Rb/E2F activity [11, 12]. MiR-449 provides a twofold safety mechanism to avoid excessive E2F-induced proliferation by cell cycle arrest and apoptosis [13]. Mathematical models have been established to explain the nonlinear dynamical behavior of the Rb-E2F pathway [13-15], which mainly concentrate on the stability and bifurcation. However, these models do not take into account the effects of noise.

Noise is ubiquitous in the real world, and always be deemed to play a destructive role in natural synthetic systems [16], but the response of a nonlinear system to a weak signal is optimized by the presence of a particular level of noise. This phenomenon is called stochastic resonance (SR) [17]. The concept of SR was firstly proposed by Benzi [18]. In the past several decades, a lot of research about SR has been performed experimentally in different fields, including biological systems [19-21], physical systems [22, 23] and so on. Moreover, theories of SR have been developed for multistable systems, monostable systems, excitable systems, as well as threshold crossing detectors [17]. Because of the coherence effect of SR on nonlinear systems, SR has been used to control harmful behavior of biological systems. In recent months, researchers at the Mayo Clinic Comprehensive Cancer Center of USA [24] discovered a way to potentially reprogram cancer cells back to normalcy and showed that restoring the normal miRNA levels in cancer cells can reverse the aberrant cell growth in laboratory experiments.

In the cell, substantial cell-to-cell variation (or

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"noise") may be observed [25], therefore, controlling the normal expression of microRNAs by using the noises and related theory becomes the primary work of this article. Firstly, the Rb-E2F pathway network involving miR449 modeled by a stochastic differential equation and their biological significance will be introduced. Secondly, by simulating and inducing the measure of stochastic resonance, how the additive noise influences the stabilities of gene network, and how to control the irregular expression of miR449 and others will be discussed. Thirdly, a mechanism to explain the biological significance of these dynamical behaviors will also be given.

EXPERIMENTAL

Stochastic differential equation model of Rb-E2F pathway mediated by miR449

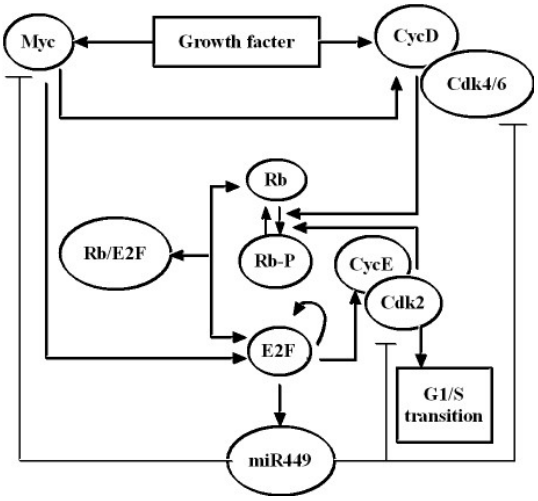


Fig. 1. Rb-E2F pathway mediated by miR449

Based on the works of Yan *et al.* [13] and Yao *et al.* [15], the simplified model considered in this paper is shown in Figure 1. Yao *et al.* [15] provided a mathematical model in the absence of miR449 and indicated that the Rb-E2F pathway acts as a bistable switch to convert signal inputs into all-or-none E2F responses. Yan *et al.* [13] dveloped another mathematical model and further investigated the stability and bifurcations of E2F,CycE and miR449 in the presence of miR449. The main task in this paper is to consider the effects of noises on the dynamic behavior of the models with miR449, when noises are taken into account. The stochastic differential equation model of the network including miR449 is described by the following system:

$$
\begin{cases}
\frac{dx_1}{dt} = k_{11}\frac{x_3}{k_{12}+x_3}\frac{x_1}{k_{13}+x_1} + k_{14}\frac{x_3}{k_{15}+x_3} + k_{16}x_4\frac{x_8}{k_{17}+x_8} + \\
k_{18}x_5\frac{x_8}{k_{19}+x_8} - k_{110}x_1 - k_{111}x_6x_1 \\
\frac{dx_2}{dt} = k_{21}\frac{x_1}{k_{22}+x_1} - k_{23}x_2 - k_{24}x_2\frac{x_3}{k_{25}+x_3} - k_{26}x_2\frac{x_4}{k_{27}+x_4} - \\
k_{28}x_2\frac{x_5}{k_{29}+x_5} \\
\frac{dx_3}{dt} = k_{31}\frac{S}{k_{32}+S} - k_{33}x_3 - k_{34}x_2\frac{x_3}{k_{35}+x_3} \\
\frac{dx_4}{dt} = k_{41}\frac{S}{k_{42}+S} + k_{43}\frac{x_3}{k_{44}+x_3} - k_{45}x_4 - k_{46}x_2\frac{x_4}{k_{47}+x_4} + \sqrt{D}\xi(t) \\
\frac{dx_5}{dt} = k_{51}\frac{x_1}{k_{52}+x_1} - k_{53}x_5 - k_{54}x_2\frac{x_5}{k_{55}+x_5} \\
\frac{dx_6}{dt} = k_{61} + k_{62}\frac{x_7}{k_{63}+x_7} - k_{64}x_6x_1 - k_{65}x_4\frac{x_8}{k_{66}+x_8} - \\
k_{67}x_5\frac{x_6}{k_{68}+x_6} - k_{69}x_6 \\
\frac{dx_7}{dt} = k_{71}x_4\frac{x_6}{k_{72}+x_6} + k_{73}x_5\frac{x_6}{k_{74}+x_6} + k_{75}x_4\frac{x_8}{k_{76}+x_8} + \\
k_{77}x_5\frac{x_8}{k_{78}+x_8} - k_{79}\frac{x_7}{k_{710}+x_7} - k_{711}x_7 \\
\frac{dx_8}{dt} = k_{81}x_6x_1 - k_{82}x_4\frac{x_8}{k_{83}+x_8} - k_{84}x_5\frac{x_8}{k_{85}+x_8} - k_{86}x_8
\end{cases}
\tag{1}
$$

where $x_1, x_2, x_3, x_4, x_5, x_6, x_7, x_8$ represent the concentrations of E2F, miR449, Myc, CycDCdk4/6 complex, CycE-Cdk2 complex, Rb, phosphorylated Rb and Rb/E2F complex, respectively, and S is the intensity of the growth factor; the noise $\xi(t)$ is assumed as a Gaussian noise with zero mean and $<\xi(t)\xi(t')>=\delta(t-t')$, and D is the noise intensity. In the following simulations, all parameter values are shown in Table 1 unless specified elsewhere.

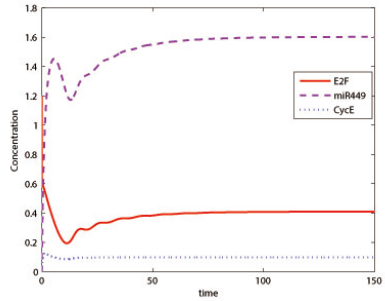
RESULTS AND DISCUSSION

Oscillation induced by noise

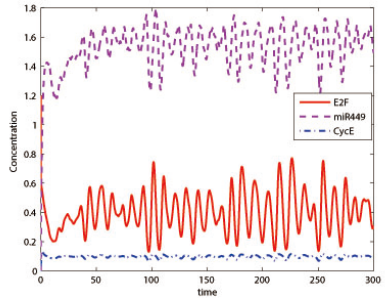
Deterministic model corresponding to the stochastic differential equation model can induce oscillation in specific parameter interval oscillation. The growth factor S was chosen as a governing parameter because it is the key parameter responsible for controlling the G1/S transition in both mammalian and plant cell cycle. Fang *et al.* [13] provided us with the following results: As the change of S, two Hopf bifurcations appear at $S \approx 1.119$ and $S \approx 3.854$ for the deterministic system. Meanwhile, when S is smaller than 0.9312 or larger than 3.854, E2F and CycE are both monostable, bistability occurs in the range starting from $S \approx 0.9312$ and $S \approx 1.117$, and sustained oscillations can be seen within the limits of $S \approx 1.119$ and $S \approx 3.854$, more details are presented in the

original article [13]. The results of numerically integrating system (1) using stochastic simulation methods, are shown in Figures 2 and 3.

Figure 2 demonstrates that noise can influence the steady states of E2F, miR449 and CycE. In Figure 2(a), the noise intensity is D=0, and E2F, miR449 and CycE are all stable. Oscillation appears when the noise intensity is D=0.1 (Figure 2(b)). In order to give a better description of the effects of noise on the genes, the results in Figure 3 show how the amplitudes of E2F, miR449 and CycE vary with the change in noise intensity under the condition of D=0, 0.001, 0.05, 0.1. It is clear that the amplitudes of E2F, miR449 and CycE are gradually increasing with the increase in D within a certain range of noise intensity.

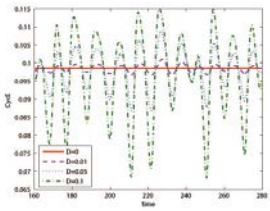


(a)

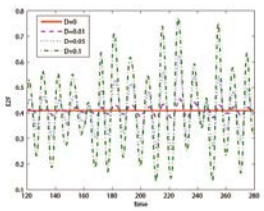


(b)

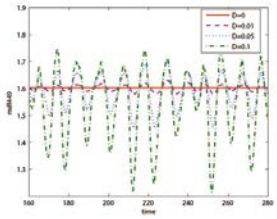
Fig. 2. Time courses of E2F, miR449 and CycE at S=5, the other parameters are shown in Table 1. Initial values are x_{10}=1.2, x_{20}=0, x_{30}=0, x_{40}=0, x_{50}=0, x_{60}=0.55, x_{70}=0, x_{80}=0, (a) D=0; (b) D=0.1.



(a)



(b)



(c)

Fig. 3. Time courses of E2F, miR449 and CycE at S=5 under the condition of D=0，0.001，0.05, 0.1. The other parameters are shown in Table 1. The initial values is as follows: x_{10}=1.2, x_{20}=0, x_{30}=0, x_{40}=0, x_{50}=0, x_{60}=0.55, x_{70}=0, x_{80}=0,

Stochastic resonance induced by noise

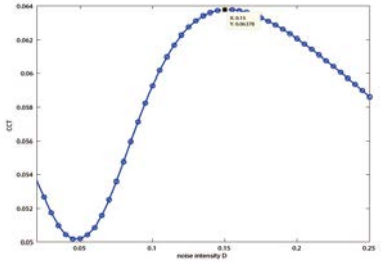
Here, the main task is to determine what is the most appropriate noise intensity for the responses of E2F, miR449 and CycE, i.e. SR. In order to effectively characterize the phenomenon of SR, the notion of the characteristic correlation time /cct is introduced as follows [26]:

$$cct = \int_0^{\infty} C^2(t)dt$$

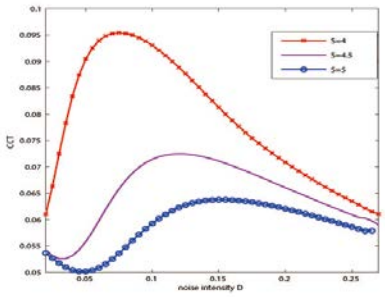
where $C(t) = < \bar{y}(t)\bar{y}(t+\tau) >$,

$$\bar{y}(t) = y(t) - < y(t) >$$

Figure 4 shows that the variation of cct depends on noise intensity, in Figure 4(a) it can be seen that cct reaches a maximum value at the noise amplitude D=0.15, i.e., SR occurs at D=0.15. According to the notion of SR above, signal responses are in the optimal state when the noise amplitude is D=0.15. Figure 4(b) shows the cct for different S (4, 4.5, 5): as the growth factor S increases, the maximal value of cct decreases, and the corresponding noise amplitude D increases.



(a)



(b)

Fig.4. Correlation time *vs* noise amplitude D: (a) S=5, (b) S=4, 4.5, 5, the other parameters are shown in Table 1.

The resonance phenomenon characterizes cancer progression [27]. Virtually, all human cancers are induced when the control of the

normal gene regulatory network is disrupted [8]. In the Rb-2F pathway, CycE and its associated kinases, cdk2 regulate the passage through the G1-phase, which together form active complexes required for entry into the S-phase [28]. Rb is the principal substrate during the G1-phase for activating cyclin/cdk complexes [29], and E2F can promote the expression of genes and entry into S-phase [30]. The numerical results suggest that E2F, CycE and miR-449 can suddenly burst even with small amplitude noise from the viewpoint of nonlinear dynamics. This small noise can cause energy to reach the energy barrier and make E2F, CycE and miR-449 away from the steady state, leading them to enter an abnormal state, resulting in the formation of a cancer cell [31].

CONCLUSIONS

Previous experimental results [25] have shown that abnormal expression of microRNAs can induce abnormal expression of its target gene, which can lead to irregular vibration of the cell and cause the cancer to happen. In the above investigation it is shown that the miR449 and its target genes (E2F, CycE, etc.) suddenly burst and oscillate irregularly when noises arise. However, when noise intensity reaches an optimal state, the oscillation of miR449 becomes increasingly regular (Figure 3), and tends to irregularity as noise intensity exceeds the optimal state. In order to explain these phenomena, a mechanism of SR is proposed, and it is found that the expression of miR449 which plays an important role in the Rb - E2F pathway can be controlled. Ref. [32] shows that the Rb/E2F pathway is critical in regulating the initiation of DNA replication and the control of this pathway is disrupted in virtually all human cancers. Also, Feng *et al.* [11,33] have shown that miR-449 regulates the Rb/E2F pathway through an auto-regulatory feedback circuit. In these complex gene networks, controlling noise intensity is a critical point in controlling cancer. According to the idea from reference [24], this paper presents a possible method to make the tumor cells to move back to normal cells through the mechanism of SR.

Table 1. Parameters for the model

Rate constant	Value	Rate constant	Value	Rate constant	Value
k_{11}	0.4	k_{32}	0.5	k_{66}	0.92
k_{12}	0.15	k_{33}	0.7	k_{67}	18
k_{13}	0.15	k_{34}	0.6	k_{68}	0.92
k_{14}	0.003	k_{35}	0.15	k_{69}	0.06
k_{15}	0.15	k_{41}	0.45	k_{71}	18
k_{16}	18	k_{42}	0.5	k_{72}	0.92
k_{17}	0.92	k_{43}	0.03	k_{73}	18
k_{18}	18	k_{44}	0.15	k_{74}	0.92
k_{19}	0.92	k_{45}	1.5	k_{75}	18
k_{110}	0.25	k_{46}	1	k_{76}	0.92
k_{111}	180	k_{47}	0.92	k_{77}	18
k_{21}	1.4	k_{51}	0.35	k_{78}	0.92
k_{22}	0.15	k_{52}	0.15	k_{79}	3.6
k_{23}	0.02	k_{53}	1.5	k_{710}	0.01
k_{24}	0.6	k_{54}	0.7	k_{711}	0.06
k_{25}	0.15	k_{55}	0.92	k_{81}	180
k_{26}	1	k_{61}	0.18	k_{82}	18
k_{27}	0.92	k_{62}	3.6	k_{83}	0.92
k_{28}	0.7	k_{63}	0.01	k_{84}	18
k_{29}	0.92	k_{64}	180	k_{85}	0.92
k_{31}	1	k_{65}	18	k_{86}	0.03

Note: the descriptions of the parameters in Table 1 are given in [13,15].

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