Journal of Theoretical and Applied Mechanics, Sofia, 2011, vol. 41, No. 2, pp. 83–92

COMBINED FIELDS

MATHEMATICAL MODELLING AND SIMULATIONS OF TUMORS, TREATED BY siRNA INFUSION*

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[Received 7 September 2009. Accepted 29 August 2011]

Abstract. A mathematical model, representing dynamics of tumor growth, suppressed by the human immune system and siRNA infusions is proposed as a system of five nonlinear ordinary differential equations. Numerical simulation of the model is carried out. It is shown that the tumor can been essentially reduced at appropriate siRNA dose, as a result of this procedure.
Key words: mathematical model, tumor growth, immune response, siRNA infusion.

1. Introduction

A big progress has been made in discovering new information and successful treatments to reduce and even clear tumors [9, 10, 11, 12], while the struggle to find an effective and permanent cure for cancer continues to challenge scientists. The immune system's apparent failure to combat many types of cancers is of fundamental importance. Tumors are derived from one or more normal cells that have undergone malignant transformation. The immune response to tumors depends on how antigenic the tumor is. A cell that has

* This work was partially supported by ESF, OP Human Resources Development, grant No BG051PO001/07/3.3-02-55/17.06.2008 and DAAD-Bulgarian National Science Fund project DOO02-23/05.03.2009.
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undergone significant mutation results in a tumor that is easier to recognize as foreign (i.e. more antigenic) than one that differs only slightly from a healthy cell. The immune response relies on some main effector cells to eliminate or slow tumor growth. Activated CD8+ T cells differentiate into cytotoxic T cells (CTLs) and can directly kill tumor cells. Phagocytic cells and natural killer cells identify and destroy tumor cells by recognizing different targets on the tumor cells than do CTLs, thus widening the scope of tumor cell killing. The strength of the immune response greatly impacts success or failure of the immune system's attack on tumor cells. In the mathematical models these dynamics are described by a positive feedback loop. Other protein modifiers are responsible for down-regulating the system (such as TGF-β, IL-10, and PGE-2). It is essential to determine ways that tumor cells are able to evade immune surveillance and methods to potentially boost tumor immunity, in analyzing the immune system's inability to clear tumors. TheTGF-β considered in this paper is not produced consistently among all tumor cells. Experiments have shown that small tumors (which receive ample nutrient from surrounding tissue) produce little or no TGF-β [1]. Most large tumors, however, secrete TGF-β and rely heavily on its growth stimulatory effects as well as immunosuppressive properties [1]. This difference helped to identify the concept that tumors can 'switch' to express immuno-suppressive properties (i.e. produce TGF-β) at a certain stage by accumulating genetic alterations that modify gene expression [1].

The theoretical study of tumor-immune dynamics has a long history. A good summary can be found in [17]. In this study however, we attempt to add to the existing literature by exploring the role of a novel medical treatment strategy known as small interfering RNA (siRNA) therapy in the tumor dynamics. In view of the pioneer character of this therapy, there are only limited published examples of such studies that tackle the kinetics of the intracellular processes, supporting by siRNA in mammals [18, 19]. Moreover, one publication connecting mathematical tools, in particular ODE modelling to siRNA therapeutic application can be found [8]. Thus, our goal is to use some of the best ideas in these studies, but to keep the model as simple as possible while incorporating the most important concepts of tumor-immune response together with the feature of siRNA therapy. We extend for the purpose a mathematical model of a cell immune response at tumor growth, presenting in a previous author paper [4] involving the influence of TGF-β production as well as siRNA infusions on tumor dynamics. We then explore the effects of the

new therapy on the model and examine under what circumstances the tumor can be reduced from an aggressive to a passive one at least.

2. Mathematical model of the cell immune response at tumor growth

A mathematical model that describes the tumor-immune cell interactions is presented by the following system of differential equations [2, 3, 4, 6]:

$$\frac{dT}{dt} = \frac{k_1 T}{1 - k_2 T} - k_3 T N - \frac{k_4 T L}{k_5 + L} \tag{2.1}$$

$$\frac{dN}{dt} = k_6 - k_7 N + \frac{k_8 T^2 N}{k_9 + T^2} - k_{10} T N \tag{2.2}$$

$$\frac{dL}{dt} = -k_{11} L + \frac{k_{12} T L^2}{k_{13} + L^2} - k_{14} T L + k_{15} T N \tag{2.3}$$

where T, N and L are tumor cells, natural killer (NK) cells and tumor-specific CD8+ T cells, respectively and $k_1 \div k_{15}$ are parameters (constants) of the model. The roles executed by NK and CD8+ T cells at the human immune response against tumor growth were shortly discussed in the introduction of this paper. The model was used in [4] to explore dynamics of tumor rejection, the specific role of the NK and CD8+ T cells, and the development of protective immunity to subsequent tumor rechallenge. Moreover, the functions describing the tumor immune growth, response, and the interaction rates, as well as associated variables, were developed using a least-squares method combined with a numerical differential equations solver. Parameter estimates and model validations used data from published mouse and human studies [13, 14, 15, 16]. Specifically, CD8+ T-tumor and NK-tumor lysis data from chromium release assays as well as in vivo tumor growth data were used in the above mentioned paper. The proposed functional forms developed demonstrated that there is a clear distinction between the dynamics of NK and CD8+ T cells. It is shown in [4] that simulations of tumor growth using different levels of immune stimulating ligands, effector cells, and tumor challenge are able to reproduce data from the published studies. In addition, an optimization procedure was carried out in the same paper and numerical values of the parameters of the system

(2.1)–(2.3) were determined. Initial numerical values of the parameters and stationary values of the variables of the model (2.1)–(2.3) were taken from the papers for the purpose [13, 14, 15, 16]. The obtained parameter values $k_1 \div k_{15}$ were used after dimensionless of the system (2.1)–(2.3) as basic ones at the solved new dimensionless model at concrete initial conditions Theoretical curves were obtained for the different kinetic variables as a result of that at different combinations of the parameter values near to the basic ones,. The theoretical obtained curves were compared with the experimental for the different kinetics variables taken from [13, 14, 15, 16]. It was seen from the carried out numerical simulation that for one concrete combination of the parameters values, the difference between theoretical and experimental values of the kinetic variables is minimal. The final parameter values $k_1 \div k_{15}$ are shown in Table 1.

Table 1. Numerical values of the parameters of the system (3.1)–(3.5)

parameter	k_1	k_2	k_3	k_4	k_5	k_6
value	4.9×10^{-1}	1.12×10^{-9}	3.51×10^{-7}	3.52	2.02×10^{4}	0.82×10^{4}
parameter	k_7	k_8	k_9	k_{10}	k_{11}	k_{12}
value	5.08×10^{-2}	2.73×10^{-2}	1.52×10^{7}	0.82×10^{-7}	2.51×10^{-1}	4.25×10^{-2}
parameter	k_{13}	k_{14}	k_{15}	μ_1	μ_2	μ_3
value	2.65×10^{7}	4.08×10^{-10}	1.41×10^{-7}	0.27	2×10^{7}	3×10^{8}
parameter	μ_4	μ_5	μ_6	μ_7	μ_8	
value	10^{6}	10^{-3}	10	5×10^{10}	0.66	

3. siRNA Treatment Model

In this section, we will extend the system (2.1)–(2.3) in order to explore an alternate therapeutic approach that utilizes siRNA strands to suppress TGF-β production. There is experimental evidence as was already mentioned in the introduction suggesting that TGF-β is produced in very small amounts when tumors are small enough. However, when the tumor population sufficiently grows, tumor cells begin to produce TGF-β in order to stimulate angiogenesis and to evade the immune response, once tumor growth resumes [1, 5]. On the other hand, the siRNA infusion considered here reduces to the following: siRNA treatment involves initial delivery of double-stranded RNA (dsRNA) into tumor cells. The enzyme Dicer then cuts the dsRNA into 21-23

nucleotide-long segments known as siRNAs that, once bound to the RNA-induced silencing complex (RISC), target TGF-β mRNA [7]. The anti sense sequence of the siRNA detects complementary mRNA strands that code for TGF-β within tumor cells and the RISC binds to and cleaves the mRNA to prevent TGF-β protein from being produced. Although not yet tested in vivo, siRNA treatment should provide a reasonable means of blocking the creation of TGF-β gene product. Ultimately, siRNA treatment works to inhibit TGF-β expression by targeting the specific mRNA sequence which leads to its synthesis, providing a possible solution to the multiple functions of TGF-β that negatively regulate cell proliferation and lead to large tumor mass. In this paper, in order to suppress this TGF-β production analogically to [8] we apply siRNA treatment. In particular, like [8] we neglect some RNA participants (as RISK and mRNAs), introducing only variables for siRNAs and TGF-β cytokines, taking into account their influence on the behaviour of other system components. It is obvious, that by introducing the last therapeutic tool connection between the intercellular and the intracellular immune response to tumor will be established. Finally, our extended model takes the following form:

$$\frac{dT}{dt} = \frac{k_1 T}{1 - k_2 T} - k_3 T N - \frac{k_4 T L}{k_5 + L} + \frac{\mu_1 T R}{\mu_2 + R} \tag{3.1}$$

$$\frac{dN}{dt} = k_6 - k_7 N + \frac{k_8 T^2 N}{k_9 + T^2} - k_{10} T N \tag{3.2}$$

$$\frac{dL}{dt} = -k_{11} L + \frac{k_{12} T L^2}{k_{13} + L^2} - k_{14} T L + k_{15} T N \tag{3.3}$$

$$\frac{dR}{dt} = \frac{\mu_3 T^2}{\mu_4 + (1 + \mu_5) S T^2} - \mu_6 R \tag{3.4}$$

$$\frac{dS}{dt} = \mu_7 - \mu_8 S \tag{3.5}$$

Notice that equations (3.2) and (3.3) are identical to equations (2.2) and (2.3), respectively. Equation (3.1) is the modified version of equation (2.1). For instance, its last term is additionally introduced to present the increased

growth of tumor cells in the presence of TGF-β and has the same form as this in an Arciero's model [8]. The equation (3.4) describes the rate of change of the extracellular concentrations of the cytokine TGF-β. The first term in equation (3.4) assumes that the production of extracellular TGF-β is inhibited as a result of the siRNA that is bound to the target TGF-β mRNA. Variable S represents total free and bound strands of siRNA, and acts as an uncompetitive inhibitor of the suppressor. The new feature is a description of how siRNA treatment suppresses the production of TGF-β, while this term still accounts for the switching mechanism that characterizes the size of cell that produces TGF-β. Equation (3.5) describes the injection and degradation of the siRNAs. In contrast to [8], however, here the delivery of siRNAs is ignored and we put the accent only on its intracellular function. Additional assumption in the model formulation is performed for that purpose, namely that the siRNA dose indirectly inhibits TGF-β production, exerting thereby on the dynamics of tumor cells. Thus, the involvement of siRNA treatment aims at least to reduce tumor growth but no totally eliminate tumor progress. The numerical values of parameters $k_1 \div k_{15}$, obtained by optimization method in [4] and additional constants $\mu_1 \div \mu_8$, taken from the current medical literature [8] are presented in Table 1.

4. Numerical simulations

In this section, a comparison between a tumor-immune model and an extended (a siRNA treatment model) will be performed by numerical simulations of both models. In particular, we will demonstrate dynamics only of tumor cells in view of the fact that they are the most important in this investigation. The numerical values of the parameters of both models are taken from Table 1. The initial values of the variables are taken from [2, 3, 6, 8] and correspond to development of metastatic melanoma cancer. In addition, in the extended model for our convenience we define constant siRNA dose ($k_{22} = 5 \times 10^{10}$ pg/ml) once a day according to [8].

The difference between the maximum tumor production (3.5×10^8 cells for the tumor-immune model and 3×10^8 cells for siRNA treatment model) is about half order at a small siRNA dose as it is shown in Fig. 1. Moreover, it is obvious, that at siRNA infusions the tumor begins to grow slower (about the 30-th day after beginning of the process) in contrast to the case when the intercellular immune system only attacks it (about the 10-th day after beginning of the process). This fact leads to the conclusion that the use of siRNAs as a

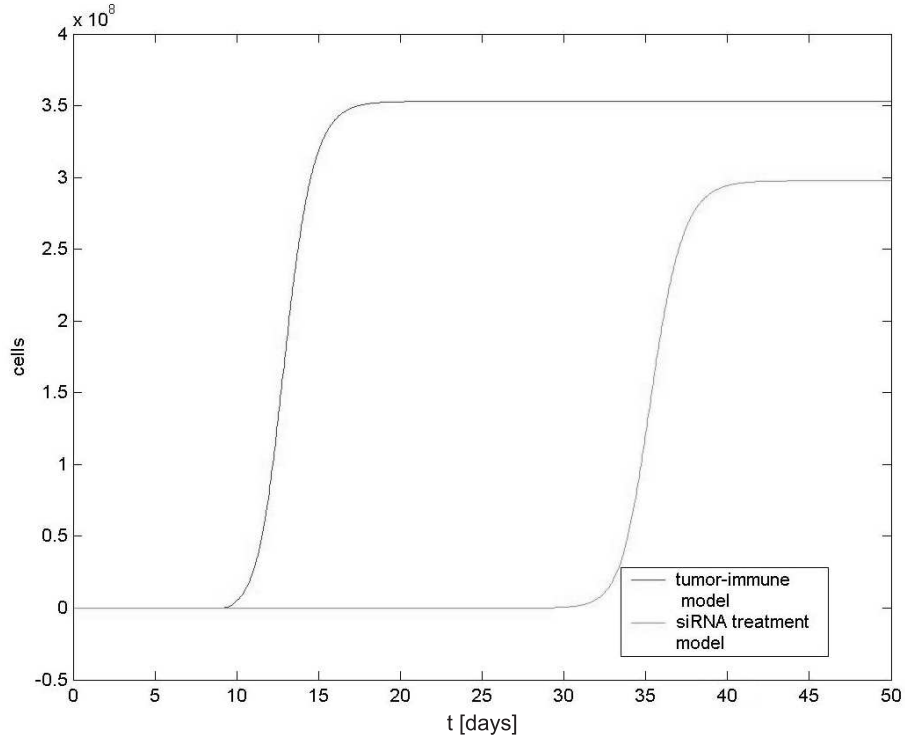

Fig. 1. Comparison between the tumor-immune model and the siRNA treatment model

medical treatment in the struggle against tumor progress will have a very positive effect. The other system component provoking the interest in this study is TGF-β. As we already mentioned TGF-β cytokines promote further tumor growth and thereby the transformation from positive to aggressive tumor will occur. The simulation of TGF-β dynamics is shown at three different siRNA doses as shown in Fig. 2. As it is seen in the same figure the maximum values of TGF-β essentially decrease at increase of the corresponding siRNA dose. This means that the tumor mass at least will stop to grow from a biological point of view, which is essential achievement in the struggle against tumors.

5. Conclusion

A well-known mathematical model in the literature of the immune response at tumor growth is extended by adding the influence of siRNA infusion in order to suppress tumor progression. Comparison between the tumor-

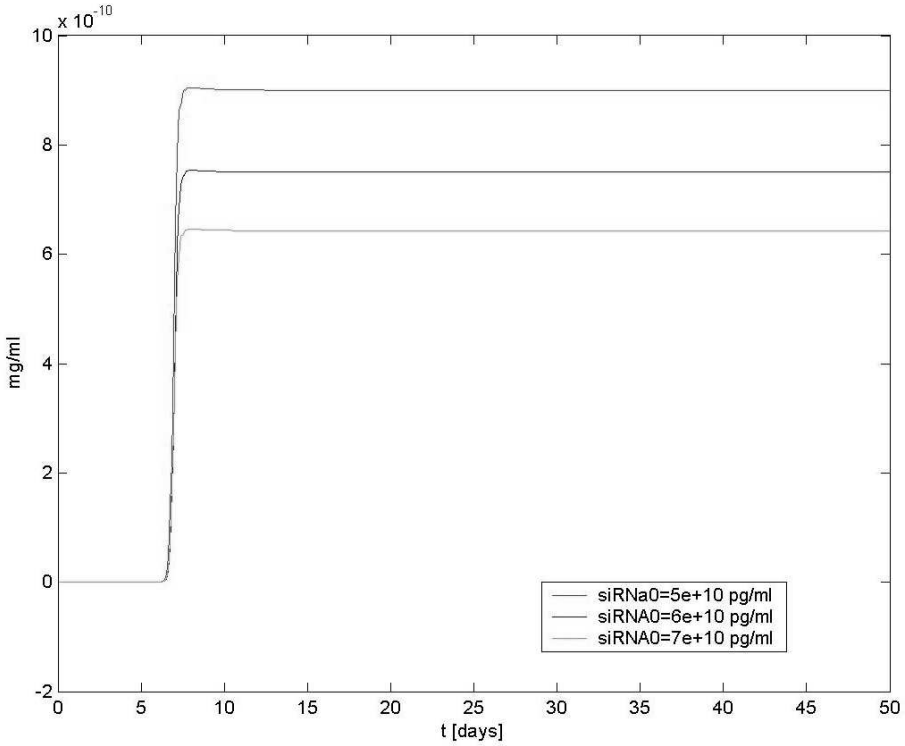

Fig. 2. Simulations of the TGF-β production at three different siRNA doses

immune model and the siRNA treatment model is carried out by numerical simulations of both models. In this way it is shown that the tumor mass reaches vastly lower maximum values at siRNA intervention than when the human immune system only attacks to tumor cells. Moreover, there is essential decrease of TGF-β production at such a treatment, responsible for further tumor growth. This fact supposes that the ill organism could be prevented at least from aggressive tumor progression.

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