Original Article

Modeling the Effect of Chemotherapy on Melanoma B16F10 in Mice Using Cellular Automata and Genetic Algorithm in Tapered Dosage of FBS and Cisplatin

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ABSTRACT

Purpose- Plans for all types of therapies for cancer need to be updated according to new achievements in science and technology. Building models of in vitro cancer cell growth may make a predictive view for physicians about the behavior of these cells in the real world.

Methods- In this study using experimental data which acquired from cultured cells and taking photos using a digital microscope lens, we designed a Cellular Automata model of death and growth of melanoma cancer cells in the presence of different concentration of FBS and different dose of Cisplatin as a chemotherapy drug.

Results- This model is based oncellular automata although we used a genetic algorithm for this model.This combined model casts a dynamic in model and made which is adoptive based on the alternation of the environment. In the end, we achieved up to 75% prediction accuracy about the behavior of these cells.

Conclusion- The proposed model showed approximately good results to predict tumor growth in the presence of different dosages of chemotherapy drug and it can make a perspective of tumor growth for us.

1. Introduction

Cancer is the name given to a cluster of about 200 diseases that presents disorderly growth of cells that invade tissues and organs [1, 2]. These cells may also spread to other tissues and can make them tumorous [3].

There is many defensive mechanisms in our body against diseases, bacteria, virus and cancer. The most important defensive mechanism of body is cytotoxic T-lymphocytes (CTL) [4]. CTLs are able to kill cells or can induce apoptosis. This ability of CTLs is compeer with a significant delay [5, 6]. This delay may be vital. Unfortunately, this delay and other deficiencies of CTLs and the escape ability of tumor cells make this mechanism fail against cancer cells. In the other words, our immune system in most times cannot overcome the cancer cells. In some studies, the dynamic behavior of CTLs against tumor cells using delay differential equations have been surveyed [7]. Because of the deficiency of the immune system against cancer cells, we need additional treatment for eliminating cancer cells. Chemotherapy is one of common alternative approach for these treatments [8]. In some studies growth of cancer cells and interactions of the immune system with cancer cells in the presence of chemotherapy drugs have been modeled and investigated [9-11].

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In this paper, we investigate a Cellular Automata (CA) model for the effect of chemotherapy using different dosages of Cisplatin and different concentration of FBS. Cellular Automata is one of useful approaches of modeling which has a wide range of applications in biological sciences such as developmental biology, neurobiology, population biology and the other fields of biological sciences [12]. In addition, there are several studies about modeling cancer growth using CA [13, 14] and in some studies the effect of time delay response of the immune system to cancer cells has been modeled using CA [6].

As we described above, for investigating the chemotherapy effect, we used Cisplatin as a chemotherapy drug. Cisplatin has a strong cytotoxic effect on cells [15]. As novelty of this paper, CA used for modeling the spatial growth of tumor cells as invitro culturing and on the other hand, in experiments Cisplatin used as chemotherapy drug which alternates tumor's growth environment, for overcoming these alternations of experiment's state, GA used as adaptive algorithm. In this paper, we present a CA predictive model for the effect of Cisplatin on melanoma cancer cells.

2. Materials and Methods

2.1. Cellular Automata

As mentioned before, our modeling approach in this study is based on Cellular Automata. In this section, we briefly introduce the Cellular Automata and later we introduce the experimental data acquisition.

Cellular Automata have become an popular approach of modeling in many sciences [16]. Cellular Automata are mathematical approaches of models for systems which have a complex nature [17]. These models use simple components with local interactions in a lattice. Each cell in this lattice interacts with its neighbors. Cellular Automata have a wide range of use from the disparate animal coat pattern of chemical reactions. Cellular Automata have helpful applications in biological systems modeling [12, 18]. The first study which used CA in cancer modeling was done in 1984 [19]. CA use n-dimensional space for their component. If this space has two dimensions, there are several definitions of neighborhood which most famous ones are listed below [20]:

1- **Von Neumann**: only North/South/East/West neighbors are considered.

2- **Moore**: one adds the diagonals. It is the case of the game of life.

3- **Extended Moore**: one extends the distance of neighborhood beyond one.

4- **Margolus**: one considers groups of 2×2. It is this type of neighborhood that is used in the simulation of gas behavior.

2.2. Experimental Data

In this section, the process of experimental data acquisition will be described and then we will introduce CA variables.

In this study, we use B16F10 cell line for investigating melanoma cancer cells. This cell line was cultured in in 96-well of culturing plate as a monolayer in controlled atmosphere (37°C, 5% CO2) using RPMI 1640. Our animal model was a mouse type C5BL/6. For pilot culturing, we used 24-well culturing plate to find the proper population of B16F10 cells in the main culturing as we needed a population that its growth and death could be traced by images of wells in a culturing plate. This experiment showed a population 6×10^4 is a proper population for our data acquisition. As Cisplatin in different dosage would be used in our experiments, we diluted this drug to 20, 10, 5, 2.5 and 1.25 µg/mL concentration.

As described above, we used 96-well of culturing plate for culturing our cell line with 6×10^4 population. These wells were divided into four groups, top left 24 wells were our first target; top right 24 wells were the repetition for the top left; the difference in the bottom left was that we introduced Cisplatin to these wells, one day later than the top wells; again the 24 wells at bottom right were a repetition for the bottom left wells. The concentrations for culturing FBS were 0, 10, 15 and 20% and the dosage for the chemotherapy drug Cisplatin were 20, 10, 5, 2.5, 1.25 and 0 µg/mL, as mentioned before.

In order to have the same quality and high resolution images of cells, we used a microscope eye-piece camera, a USB 5 MP 23 mm model from Dino-Lite. The minimum magnification we could get from the combination of microscope and the eye-piece camera did not let us have a whole image from each cell. We had to choose the most proper point of each cell and trace it afterwards. To be sure we were tracing the same point each time we got images; we used a Plexiglas plate under the culturing plate which was scaled into 1mm×1mm squares by means of laser graving.

For a full week after the cultured cells were ready, we took two images, each day from a particular point in each well, first in the morning and the second in the afternoon. After the first week, all cells were treated with alcohol and were disposed.

As decided before, the main program needed the images in binary and the space occupied by the cells was considered as "1" and the remained space as "0". Edge detection and segmentation had to be applied to the input images. This task was done by cropping, making gray scales, applying the Sobel operator and threshold, dilating using linear structuring elements, filling the holes and blurring, and at last inverting, respectively.

As described in section 2.1, there are many types of CA for modeling systems. In this study, we used Moore neighborhood for our CA model.

3. Results

As described before, we divided the culturing plate into four areas, theoretically. There are top left, top right, bottom left and bottom right. All 96 wells were traced for a full week and their images were captured twice a day and totally 1300 images were taken. In this section, we selected one well from each group of wells randomly for illustrating results. The wells number is included C9 (33), D1 (37), E10 (58), and F6 (66). C9 (33) in the top right contains 2.5 µg/mL Cisplatin and 15% FBS; D1 (37) in top left contains 0 µg/mL Cisplatin and 20% FBS; E10 (58) in bottom right contains 5 µg/mL Cisplatin and 0% FBS; and F6 (66) contains 20 µg/mL Cisplatin and 10% FBS. One sample of these wells is illustrated in Figure 1.

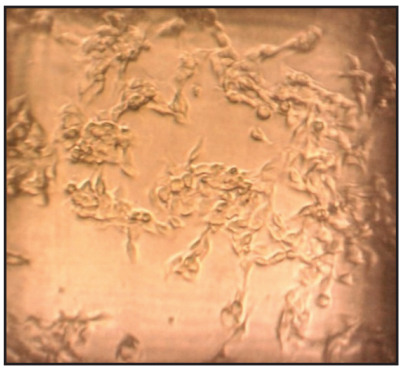

Figure 1. One sample photo taken from B16F10 melanoma cancer cells cultured in a 96-well culturing plate. Captured by means of a microscope eye-piece camera, a USB 5 MP 23 mm.

In Figure 2, the block diagram of modeling approach for our study is illustrated. The initial image added by 5% noise, considered as the input of the model, then the manner of tumor cells modelled as 2D CA with 8-n neighbourhood and then the output of the model at each step compared with the real image using GA. The manner of tumor cells which considered in this model consists of proliferation, apoptosis, movement and quiescence. Proceeds of CA are updating using GA. The parameters of CA which are updating using CA consist probabilities of proliferation, apoptosis, movement and quiescence. In the first iteration, possibilities of each event considered as random variables, but at next iterations, these values optimized using GA. For using GA, an initial population needed, in this model the initial population considered as 16. This population may be increased to achieving better results and less error, but this population increasing may increase runtime and would be time consuming.

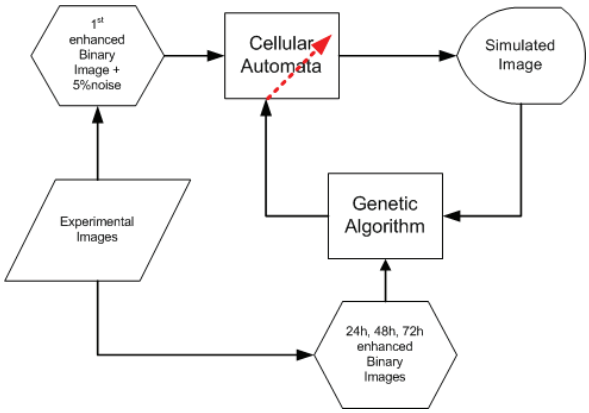

Figure 2. Block Diagram of model.

In every calculation in Cellular Automata combined with a Genetic Algorithm, a minimum cost is illustrated in the output to show the rate of correlation. The cost function which used in GA was the rate of correlation between images of experiments an output images of model that described in equation (1). In Figure 3 a sample figure created by the MATLAB software for well D1 (37) is shown.

Where X is experimental image and Y is output image of the model, i and j is coordination in images and C is correlation between X and Y. The GA adopted the parameters of the CA and decreased the difference between our model's output and experimental data. At each step, the GA prevent the error accumulation in model. Finally the model was corrected by GA and

showed better results which matched to the experimental data as much as possible. GA adopted parameters such asthe viability of cancer cells, possibility of proliferation, possibility of movement, possibility of apoptosis and the other parameters.

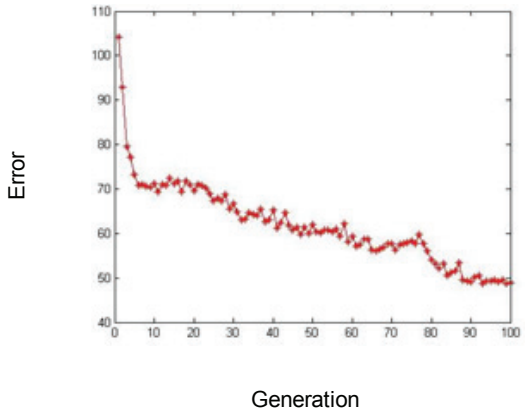

Figure 3. One sample diagram of minimum cost calculated by Cellular Automata combined with a Genetic Algorithm in MATLAB for an iteration of 100 times.

In the following, the results of the main program which include an image enhancement, a Genetic Algorithm and a Cellular Automata are illustrated. In Figure 4, the result of well C9 (33) is shown.

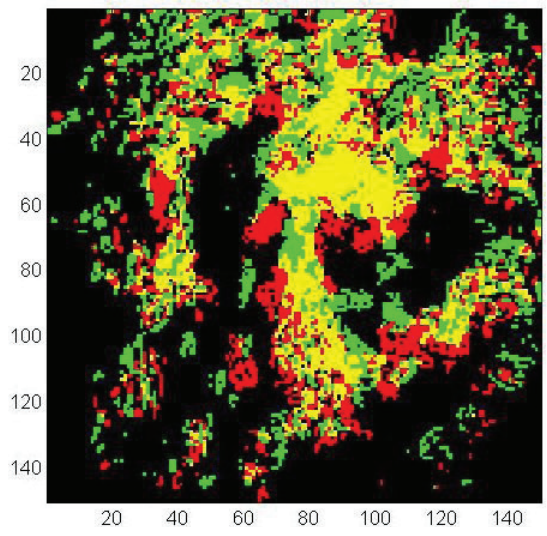

Figure 4. One sample result from well C9 (33) created by MATLAB as the final output for the main program which contains an image enhancement, a Genetic Algorithm and the Cellular Automata.

Each image of the cells was considered as 150×150 matrix in this program. As described in experimental data acquisition, the images of data captured using 5 Mpixels camera. In model 150 × 150 pixels matrixes were used. This low resolution matrix in comparison with high resolution image acquisition camera was made for making less complicity model and, on the other hand, changes in data could be shown in 150×150 pixels matrix. In calculations, the whole image was considered as a single area as we did not get better results when dividing the whole area into more areas. As illustrated in Figure 4, a result was obtained by correlating the final cell image with the Cellular Automata output to have a better understanding of the program success in predicting the cell behavior. The final image of cells is shown in green color, the model output is shown in red, and wherever the model correlates with real image is a combination of green and red colors, a yellow color.

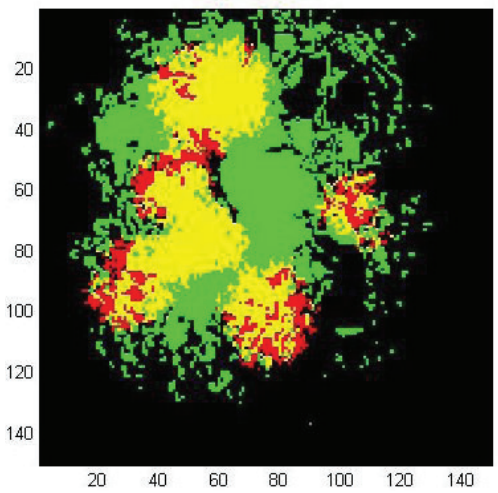

Figure 5. One sample result for well D1 (37) created by MATLAB as the final output for the main program which contains an image enhancement, a Genetic Algorithm and the Cellular Automata.

Figure 5 shows another output of the model. As it may be seen, the zero amount of chemotherapy drug with an FBS of 20% has made a good environment for the cells to grow and fulfill the well. Some of the parameters used in the model are variables that the Genetic Algorithm assists the Cellular Automata for obtaining the best value of them, which results in a more precise output, athough some of the parameters are constant during the program. The most important parameters are the drug dosage, the dimension of matrices, the viability of cancer cells, the possibility of proliferation, the possibility of movement, the possibility of apoptosis, cells populations, and a few more which depends on the programming techniques skill and philosophy.

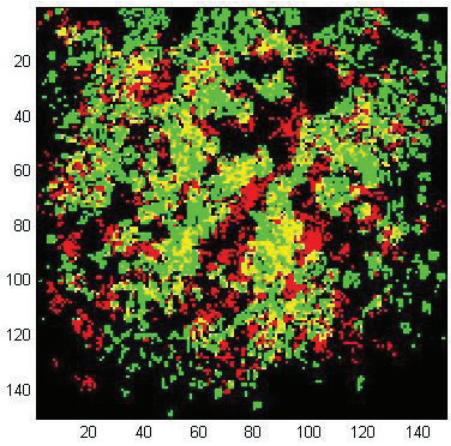

Figure 6. One sample result for well E10 (58) created by MATLAB as the final output for the main program which contains an image enhancement, a Genetic Algorithm and the Cellular Automata.

Figure 6 is another output of the created model. It is obvious that cells cultured in a zero percentage of FBS and 5 μg/mL of Cisplatin are not going to make tumor-like colonies.

In Figure 7, the result of CA for well F6 (66) has been illustrated.

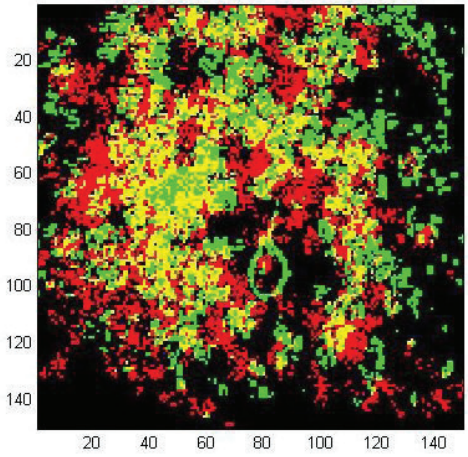

Figure 7. One sample result for well F6 (66) created by MATLAB as the final output for the main program which contains an image enhancement, a Genetic Algorithm and the Cellular Automata.

Whilst capturing images during the experiment, it was very important to make the microscope focused. This is because at the time of cell death, it leaves the bottom of the well and moves to the surface, an improper focus makes the image a mess.The outputs of the model showed approximately matched results to the experimental data, this matching factor evaluated using a correlation between images of experimental data and output images of the model. This matching factor, evaluated for all 96-well and results showed 75% matching with 2% standard deviation, without using GA, this matching factor was 47% with 8% standard deviation. These results showed the efficiency of GA application in CA model.

4. Discussion

In this study, we presented a CA model which can represent a prediction for cancer cells growth. This prediction can introduce a complicated behavior of melanoma cancer cells. Our results in this model showed that using CA based models for modeling complicated world, especially in biology may have better results than the classic models. One of the advantages of CA models is the inclusion of time and location. In the other words, CA models have either temporal and spatial resolutions and this advantage makes the ability of summarizing the rules in spatial and temporal directions. In this study, our first aim was not to achieving quantitative results, but to achieving qualitative results.

For making the mouse cancerous, we used B16F10 cell line and these cells were in the presence of Cisplatin and FBS in different dosages. Our model has almost a similar output. This similarity between experimental data and the output of the model was approximately 75%±2%. This similarity between models'output and experimental data was achieved using GA to prevent error accumulation. This similarity was not suitable without GA and the model has less similarity to experimental data. As described above, this model's goal is the prediction of melanoma cancer cells'behavior to have better insight about the effect of Cisplatin as a chemotherapy drug and environment effect on this tumor growth, therefore better similarity may provide better predictions. The lack of information about variables and the initial condition is a major cause of decrement of similarity between the model and experimental data. The increment of experimental data and repetition of experiments do not necessarily guarantee the best results, but it may help create a better description of the relation between parameters of the model. We believe that the new progression in science and technology is where two sciences have met; this is obvious as our work is a combination of immunology knowledge with engineering techniques.

It is helpful to use numerous magnifications in taking photos of the cell to have a better understanding of their behavior. For future work, a 3D model with a more precise signaling description between the cells will make a step forward to the real in vivo environment. Ideal models can be implemented in cancer treatment plans in near future.

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