DNA-based Killer Automaton: The Innovative Nanomedicine

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ABSTRACT

In this paper, we propose the DNA-based Killer Automaton (DKA), an innovative nanomedicine for cancer treatment. Equipped with an internal DNA Computing algorithm, our DKA detects cancer by checking the mRNA strands inside the cells. If a cancer cell is detected, cytotoxic materials are released by the DKA to eliminate the cell. In addition, due to the *bystander* effect, cytotoxins carried by the DKA can propagate only to cells that express cancerous behavior, thus having the potential for completely curing the cancer with minimal side effects.

Keywords: DNA Computing, the *bystander* effect, nanomedicine, DNA Automaton, Gap Junction Intercellular Communication (GJIC)

1 INTRODUCTION

As an emerging technology, DNA computing [1] is becoming increasingly important in many different scientific fields, including supercomputing, biology, chemistry, medicine, etc. Recently, Shapiro has proposed utilizing the computing power of DNA/RNA to diagnose and cure genetic mutation diseases such as cancer [2]. However, Shapiro's DNA automaton does not really cure cancer; instead, it blocks cancer expressions at the translation level by utilizing DNA antisense.

Based on Shapiro's molecular automaton model, we propose an alternative DNA automaton model, the DNA-based Killer Automaton (DKA), which has the potential to completely remove the cancer cell line. DKA has two advantages that make it a great candidate for future cancer therapies. First, unlike the antisense therapy which blocks the translational expressions of cancer cells, DKA has the potential of killing all cancer cells, therefore completely curing the patient of the disease. Second, unlike other therapies that introduce serious side effects, DKA selectively targets the mutation site without massively destroying any nearby healthy cells, thereby minimizing side effects.

In this paper, we explain how DKA detects and cures cancer with great efficacy and minimal side effects. Also, we present a software model that simulates the DKA mechanism in an artificial multi-cell environment.

2 BACKGROUND

We based this project on the fundamental work of Shapiro, who initially described an automaton capable of infiltrating and detecting cancer cells, and some discoveries related to the interactions (or rather lack of) between cancerous cells and healthy cells. These fundamental research projects are now summarized prior to describing our advances.

2.1 Shapiro's Molecular Automaton

Shapiro's automaton contains three major parts: an antisense [3] DNA single strand, which is folded into a hairpin structure; a cancer detector, which is a DNA double strand that locks the antisense DNA single strand; and restriction enzymes, which are used to cut the cancer detection sites during cancer detection. When a cell becomes cancerous, its mRNAs express cancerous behavior and thus contain different cancer indicators [4]. If a series of cancer indicators are detected, then it is very probable that the cell is cancerous. The cancer detector in Shapiro's model is composed of a double-stranded DNA which has a short single-stranded DNA (ssDNA) attached to its left side. The double-stranded DNA has two parts: the upper single strand contains different DNA segments that are complementary to a series of mRNA cancer indicators while the lower part locks the upper part so that they cannot function until needed. The ssDNA is a complementary strand to the first cancer indicator in mRNA. If the first indicator binds to the ssDNA, a restriction enzyme FOKI cuts the binding site as well as the lower part of the next DNA segment, thus opening the upper part of the next DNA segment. Then, this new single-stranded DNA segment can check whether the second cancer indicator exists in cellular mRNA. By repeating this process, if all cancer indicators have been detected and all segments of the cancer detector have been cut, then the antisense DNA hairpin is released and unfolded, and consequently it functions as a drug that blocks the cancer expression in cellular mRNA.

2.2 The *Bystander* Effect

The *bystander* effect is a biological phenomenon observed in suicide gene therapy [5]. In suicide gene therapy, cytotoxins, such as ganciclovir triphosphate (GCVTP), are produced in some of the cancer cells (host cancer cells). In addition to killing these host cancer cells by inhibiting DNA synthesis in the S cell cycle, GCVTPs are able to

propagate to neighboring non-host cancer cells, and therefore, exert toxic effects on these neighboring cancer cells as well. This propagation of cytotoxins due to the *bystander* effect is very effective. Indeed, it has been shown that if GCVTPs initially enter as few as 10% of the cancerous cells, they can result in complete tumor regression after propagation [6]. Homologous gap junctional intercellular communication (GJIC) is the main contributor to the *bystander* effect [7]. They are protein channels (connexins) connecting cytoplasm of cells in the same cell line so that they enable a direct diffusion from cytoplasm to cytoplasm for chemicals with molecular weight less than 1000 Daltons [8]. Homologous GJIC channels do not exist between cells from different cell lines.

3 DNA-BASED KILLER AUTOMATON

As Figure 1 shows, our DKA destroys cancer malignancies in three steps: cancer detection, cytotoxin propagation, and programmed cancer cell death. Similar to Shapiro's molecular automaton, our DKA detects cancer by checking mRNA strands in cells. However, when cancer is detected in a cell, instead of releasing DNA antisense to block cancer expressions at the translation level, the DKA releases cytotoxin to kill the cancer cell. In addition, the *bystander* effect increases the efficacy of DKA so that DKA has the potential to result in complete tumor regression as long as they enter 10% of cancer cells.

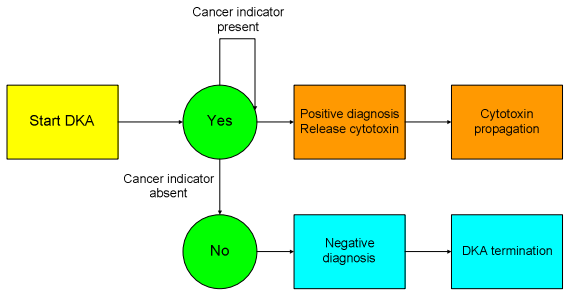

Figure 1: Algorithms of DNA-based Killer Automata

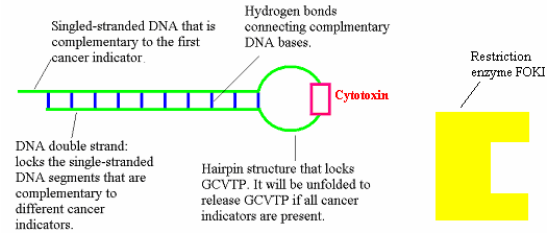

Figure 2: DKA structure

As Figure 2 shows, DKA has a structure similar to Shapiro's DNA automaton. However, instead of DNA antisense, cytotoxin is placed in the hairpin-structured "drug" section. Like Shapiro's model, DKA detects cancer by using the algorithm shown in Figure 3. When DKA enters a cancer cell, the single-stranded DNA portion of the automaton binds to its complement, the first mRNA cancer indicator. Next, the restriction enzyme FOKI recognizes the binding site and cuts it, unlocking the second single-stranded DNA segment that is complementary to the second cancer indicator on the mRNA strand. Following the same steps, if all cancer indicators are detected one after the other, and then the last segment of the DNA double strand is cut, thus releasing cytotoxins to kill the cell.

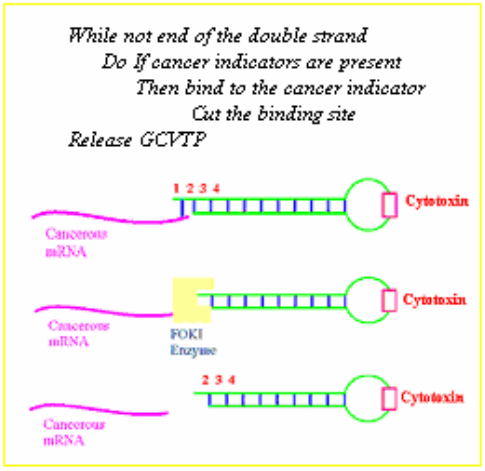

Figure 3: DKA cancer detection algorithm

Due to the *bystander* effect, any cytotoxin excess released inside a cancer cell propagates to neighboring cancer cells through homologous GJIC channels, eliminating neighboring cancerous cells as well. As mentioned above, the cytotoxin excess cannot propagate to healthy cells due to the lack of GJIC channels, thus minimizing side effects. In addition, if a DKA accidentally enters a healthy cell, it does not harm the cell due to the lack of cancer indicators to unlock the cytotoxin.

The main challenge faced by this model is that restriction enzymes are not usually present in mammalian cells. If it was introduced to a mammalian cell, it might cut random mRNA strands in a healthy cell. This therefore, prevents DKA from working *in vivo* at this stage. One possible solution is to deliver this enzyme to cancer cells in DNA form with a mammalian expression promoter attached. In this paper, we focus on the software simulation of the DKA mechanisms based on the assumption that this challenge has been overcome.

4 TOOLS AND METHODS

By utilizing Object Oriented Programming techniques, we have constructed a software model to simulate the DKA mechanism in an artificial multi-cell environment. The main tool we used for this simulation is the Sun Java 2 Platform Standard Edition Software Development Kit (SDK). In this simulation, each component of DKA is constructed as a Java object and assigned specific chemical and physical properties. For instance, the mRNA objects are assigned base-pair complementarities such that base C

would be able to bind to base G. Figure 4 shows the architecture of the DKA simulation software: the top level object Experiment describes the interactions between DKA and cells and it is supported by the underlying objects.

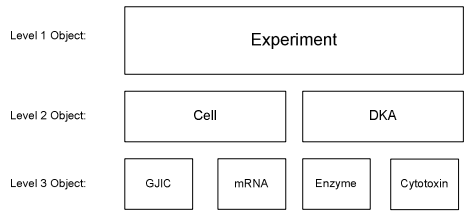

Figure 4: Simulation software architecture

We simulated the DKA mechanisms in three stages: distributions of DKA, cancer detection, and cytotoxin propagation. For distributions of DKA, we assume that a maximum of 100,000 DKAs (0.1% of the cell weight) can enter one cell without harming the cell. Therefore, the program generates a random number (which ranges from 0 to 100,000) of DKAs to enter each chosen cell until all DKAs have been distributed. After entering the Cell object, the DKA objects detect whether the host cell is cancerous or healthy by checking the host cell's mRNA objects using the detection algorithm mentioned before. If cancer is diagnosed in the host cell, then cytotoxin objects are released. Otherwise, the DKA object terminates. At last, these cytotoxin objects propagate from one cancer cell to another through the GJIC channels. There is an equilibrium condition at which all cytotoxin objects stabilize and are no longer able to propagate. In this program, we define the threshold for disrupting the equilibrium condition as a difference of 10,000 DKA (0.01% of the cell weight) between two neighboring cancer cells. After the equilibrium condition has been reached, the program counts the number of cytotoxin objects in every cell so that the cells that contain cytotoxin objects are eliminated.

5 EXPERIMENTS AND RESULTS

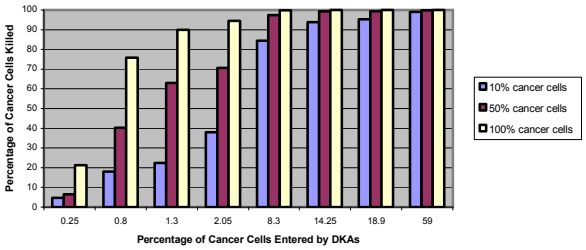

Figure 5: Simulation result I.

In this simulation, we create three different cell groups: 1,000 cells in which 10% are cancerous, 1,000 cells in which 50% are cancerous and 1,000 cells in which 100% are cancerous. In each of these three cases, we conduct 20 different experiments by adjusting the number of DKAs used. For each experiment, we record the percentage of cancer cells that are initially penetrated by DKAs. Also, we record the percentage of cancer cells that are killed by cytotoxins after the cytotoxin propagation stage. To make sure that the results are reliable, we repeat each experiment 100 times and take the average value as the final result.

Figure 5 shows the percentage of cancer cells killed versus the percentage of cancer cells entered by DKA initially. For 1,000 cells in which 10% are cancerous (blue bar), this results in >95% tumor regression when DKAs have initially entered more than 18% of cancer cells. For 1,000 cells with 50% cancer cells (red bar), there is >95% tumor regression when DKAs have initially entered more than 7.9% of cancer cells. Similarly, for 1,000 cells with 100% cancer cells (yellow bar), there is >95% tumor regression when DKAs have initially entered more than 2.1% of cancer cells. This confirms that DKAs do not have to enter all cancer cells in order to destroy cancer malignancies. In addition, this demonstrates that there is a relationship between tumor regression and the cancer cell percentage such that a higher cancer cell percentage results in a higher tumor regression. This finding is reasonable because a higher cancer cell percentage implies a higher density of homologous GJIC channels, and consequently a better spread and propagation of cytotoxins. Particularly, in the 50% cancer cell case, the threshold percentage for complete tumor regression is 10.5%, which is in perfect agreement with the data obtained from suicide gene therapy experiments with 50%-50% cancerous to healthy cell culture [6].

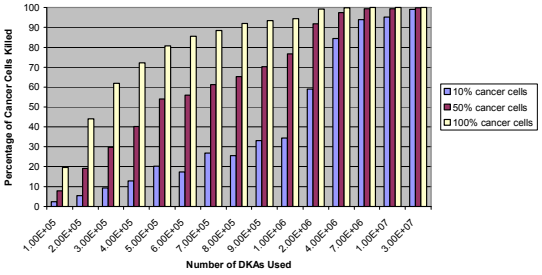

Figure 6: Simulation result II.

Figure 6 shows the percentage of cancer cells killed versus the number of DKAs used. As expected, the drug efficacy increases as the number of DKAs (dose) increases. Furthermore, it requires different doses for cell lines with different cancer cell densities to achieve >95% tumor regression. The simulation results show that the doses required to result in >95% tumor regression for 10%, 50%, and 100% cancer cell density in a cell line with 1000 cells are 5,000,000, 3,000,000, and 1,000,000 DKAs, respectively. This piece of information clearly shows that the percentage of cancer cells killed is directly related to the number of DKAs used (drug dosage) and the cancer cell percentage

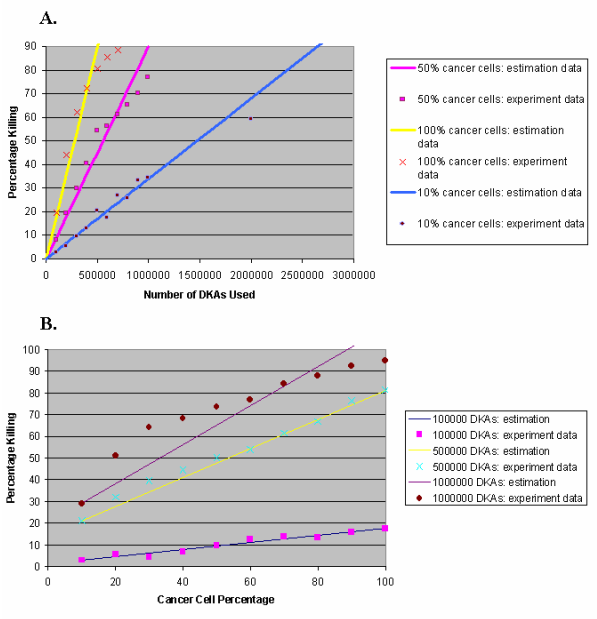

Figure 7: DKA's cancer cell killing efficacy

To identify the relationship between the efficacy of the DKA approach, the cancer cell percentage, and the number of DKA injected into the cell group, we have organized the simulation results in Figure 7 and added estimation trend lines for these pieces of data. From Figure 7, we conclude that for a fixed cancer cell percentage the efficacy of DKA linearly depends on number of DKA injected into the cell group before saturation. After reaching saturation, increasing the number of DKAs has little effect on the killing efficacy. On the other hand, for a fixed number of DKAs used, the efficacy of the DKA approach linearly depends on the cancer cell percentage. Therefore, the efficacy of DKA (E) linearly depends on the cancer cell percentage (D) and the number of DKA injected into the cell group (N). It can be described by equation 1.

$$E = const. \times D \times N \qquad (1)$$

6 CONCLUSION AND FUTURE WORK

This paper has proposed the DNA-based Killer Automaton, an innovative intelligent nanomedicine which has the potential of completely curing cancer with minimal side effects. In order to predict the efficacy of this approach, we have conducted software simulations to simulate the DKA mechanism. The simulation results present in this paper have demonstrated that DKA is very efficient in curing cancer due to its ability to detect and cure cancer in cells, and propagate toxicity throughout the cancer cell line. As the results indicate, DKAs do not have to enter all cancer cells in order to result in complete tumor regression, depending on the density of homologous GJIC channels, as long as DKAs can enter a certain percentage of all cancer cells, they are able to propagate to all cancer cells through due to the *bystander* effects. In addition, by analyzing the simulation results, we have discovered that the efficacy of this nanomedicine is linearly dependent on the number of DKAs injected into the cell group and the density of homologous GJIC channels.

The next stage of our research is to construct a more sophisticated mathematical model to describe the delivery of DKA in tumors. For this mathematical simulation, we plan to utilize and modify the Virtual Tumor simulator (constructed at the UC Irvine Center of Computational Science of Microstructure) so that the *bystander* effect can also be described mathematically.

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