Author

Prior to beginning his research, Shaoshan Liu had developed a bond with Professor Gaudiot in many discussions developing research ideas. When Shaoshan began formulating his proposal for DNA computing, he discussed this with Professor Gaudiot who recognized both the value of the research and Shaoshan's unique approach, and invited him to join the research in his lab. Shaoshan's research is truly multidisciplinary—combining computer science, biology, medicine and chemistry—and it is this combination that is truly exciting. Shaoshan plans to continue with multidisciplinary research, by first pursuing a Masters degree in EECS, followed by a Ph.D. in Engineering.

Key Terms

- Artificial Multi-Cell Environment
- *Bystander Effect*
- DNA Automaton
- DNA Computing
- Gap Junction Intercellular Communication (GJIC)
- Nanomedicine
- Object-Oriented Programming
- Restriction Enzyme

Software Simulation of DNA-Based Killer Automaton: The Innovative Nanomedicine

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Abstract

The DNA-based Killer Automaton (DKA) is an innovative nanomedicine for cancer treatment. Equipped with an internal DNA computing algorithm, DKA detects cancer by checking oncogenic mRNA sequences in cells. If these cancer markings are detected, DKA releases cytotoxic materials to destroy the cancer cell. In addition, due to the *bystander effect*, the cytotoxic materials are able to propagate only to the cells that express cancerous behavior, thus destroying the malignancy with minimal side effects. To predict the efficacy of DKA in cancer treatments, a software model has been created to simulate the DKA mechanisms in an artificial multi-cell environment. The results obtained from the simulations show that the efficacy of DKA is linearly dependent on both the amount of DKA injected into the cancer cell group and the density of homologous gap junction intercellular communication (GJIC) channels. Also, the results verify that DKA does not have to enter all cancer cells to destroy malignancies. Depending on the density of homologous GJIC channels, DKA can enter a certain percentage of cancer cells, and propagate to all other cancer cells through the *bystander effect*. The result is complete tumor regression.

Faculty Mentor

DNA is not only the fundamental information carrier of life, it can be used to perform logic functions as well. In 2004, Ehud Shapiro constructed a DNA computer model capable of diagnosing cancerous activity within a cell. Shaoshan's project extends Shapiro's model to the point where the DNA computer can not only diagnose cancerous activity in cells but also (at least theoretically) eliminate the cancer cells and leave healthy cells untouched. Shaoshan's fascinating work required creativity, scientific curiosity, motivation, diligence, and the integration of knowledge at the crossroads between computer science, nanotechnology, and cancer biology. It sets a perfect example of where interdisciplinary research can lead.

Jean-Luc Gaudiot
Henry Samueli School of Engineering

Introduction

Cancer, a type of disease characterized by uncontrolled growth and spread of abnormal cells, has a very high fatality rate. It is a leading cause of death in the U. S., following only heart disease. According to estimates by the American Cancer Society (Cancer Facts and Figures 2005), 570,280 Americans are expected to die of cancer this year, more than 1,500 people every day. This constitutes one out of four deaths in the U. S. The National Institutes of Health estimated the overall cost of cancer treatments exceeded $69.4 billion for 2004 (Cancer Facts and Figures 2005). Unfortunately, even with this level of financial commitment, major cancer therapies, including surgery, radiation, chemotherapy, hormones, and immunotherapy, cannot always accurately target cancerous cells, and thus often introduce more lethal side effects and/or fail to provide a cure. While an effective cure has remained elusive, new methods drawn from fields such as nanotechnology and DNA computing may hold the key for novel approaches.

Recently, Benenson *et al.* have proposed using the computing power of DNA/RNA to diagnose and cure genetic mutation diseases such as cancer (2004). The basic idea of this approach is that, by checking mRNA strands in cells, DNA automata can detect the presence of cancer indicators and release single-stranded DNA cancer suppressors (DNA antisense) if all relevant cancer indicators have been detected. One major disadvantage to this approach is that the DNA automaton does not cure cancer; instead, it blocks cancer expressions at the translation level by using DNA antisense. This requires cancer patients to take the expensive DNA-automaton medicine periodically, which may not be practical.

We propose an alternative automaton model, the DNA-based Killer Automaton (DKA), which has the potential to completely destroy a malignancy in one treatment. DKA has two advantages that make it a strong candidate for cancer therapy. First, unlike the antisense therapy, which blocks the translational expressions of cancer cells, DKA has the potential to eliminate all cancer cells, completely destroying the malignancy. Second, unlike other therapies that introduce serious side effects, DKA targets the mutation site without damaging nearby healthy cells, which minimizes side effects. This paper explains how DKA detects and destroys malignancies with great efficacy and minimal side effects, and presents a software model that simulates the DKA mechanism in a multi-cell environment.

Background

This project is based upon the fundamental work of Benenson *et al.*, who initially described a molecular automaton model (MAM) capable of infiltrating and detecting cancerous cells. It also takes discoveries into account that are related to the interactions between cancerous cells and healthy cells. The MAM applies antisense cancer gene therapies, which are based on the identification of oncogenes and aim to suppress the expression of specific oncogenes (Jansen et al. 2002). Antisense oligonucleotides are short single-stranded DNA (ssDNA) that are complementary to cellular mRNA that express cancer. In cells, antisense DNA binds to oncogenic mRNA and thereby hinders abnormal cell growth. The MAM contains three major parts: an antisense ssDNA, which is folded into a hairpin structure; a cancer detector, which is a double-stranded DNA (dsDNA) that locks the antisense ssDNA; and restriction enzymes, which are used to cut at cancer detection sites (Figure 1).
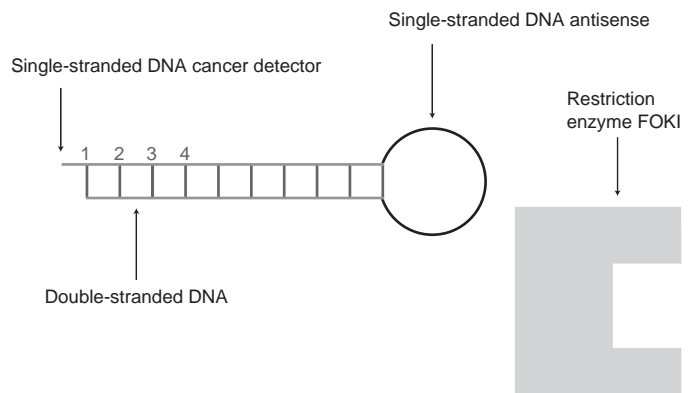

Figure 1
Benenson *et al.'s* Molecular Automaton: this automaton is composed of three parts: 1) a DNA cancer detector, 2) a restriction enzyme that cuts the cancer detection site, and 3) a DNA antisense that blocks cancer expression after cancer is detected.

When a cell becomes cancerous, its expressed mRNA exhibits cancerous behavior and thus contains different cancer markers (Sidransky 2002), which are RNA short strands that are 7-base long. The cancer detector in the MAM is composed of a double-stranded DNA that 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 the upper segments cannot function until needed. The ssDNA is complementary to the first cancer indicator in mRNA. If the first indi-

cator binds to the ssDNA, the restriction enzyme FOKI (Blanche et al. 2002) cuts the binding site and the lower part of the next DNA segment, opening the upper part of the next DNA segment. This new single-stranded DNA segment checks whether the second cancer indicator is present in cellular mRNA. By repeating this process, if all cancer indicators have been detected, all segments of the cancer detector will have been cut, and the antisense DNA hairpin is released and unfolded. Consequently, the automaton functions as a drug that blocks the oncogenic expression in cellular mRNA.

The Bystander Effect

The *bystander effect* is a biological phenomenon observed in apoptotic gene therapy, in which cytotoxic materials, such as ganciclovir triphosphate (GCVTP), are produced in some of the host cancer cells (Pitts 1994). In addition to killing these host cancer cells by inhibiting DNA synthesis in the S cycle, GCVTP is able to propagate to neighboring non-host cancer cells, exerting toxic effects on these cells as well. This propagation of toxicity through the *bystander effect* is extremely effective. Indeed, it has been shown that if GCVTP enters as few as 10% of cancerous cells initially, it can propagate and cause complete tumor regression after propagation (Culver et al. 1992).

Homologous gap junctional intercellular communication (GJIC) is the main contributor to the *bystander effect* (Yang et al. 1998). As Figure 2 shows, homologous GJIC channels are protein channels (connexins) that connect the cytoplasm of cells in the same cell line, so that direct diffusion from cytoplasm to cytoplasm can occur (Duflot-Dancer et al. 1997). GJIC channels are able to transport chemicals with a maximum molecular weight of 1,000 daltons (Da) (Sompson et

al. 1977). Since the molecular weight of GCVTP is 496 Da, GCVTP is able to propagate to neighboring cells through GJIC channels. In cells, GCVTP does not inhibit eukaryotic cell polymerases, and can thus be used as a substrate for DNA synthesis. Incorporating GCVTP into the DNA of dividing cells results in DNA chain termination and, consequently, cell death.

Proposed Model: DNA-Based Killer Automaton

DKA combines the MAM developed by Benenson *et al.* with the apoptotic gene therapy *bystander effect*. It destroys cancer malignancies in three steps: cancer detection, toxicity propagation, and programmed cancer cell death (Figure 3). Similar to the proposed MAM, DKA detects cancer by checking mRNA strands in cells. However, when cancer indicators are detected in a cell, instead of releasing DNA antisense to block oncogenic expressions at the translational level, DKA releases cytotoxic materials to kill the cancer cell. As a result of the *bystander effect*, the efficacy of DKA is increased so that it has the potential to cause complete tumor regression.

Figure 3
Algorithm of DNA-based Killer Automata

The structure of DKA is similar to that of the MAM (Figure 4). However, instead of DNA antisense that blocks translational expression, cytotoxic material GCVTP is placed in the hairpin-structured "drug" section. As with the MAM, DKA uses the algorithm in Figure 5 to detect cancer. When DKA enters a cancer cell, the single-stranded DNA portion of the automaton binds to the first cancer indicator on the mRNA strand. 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. Likewise, if all cancer indicators are detected, the last segment of the DNA double strand is cut, releasing GCVTP to kill the cell.
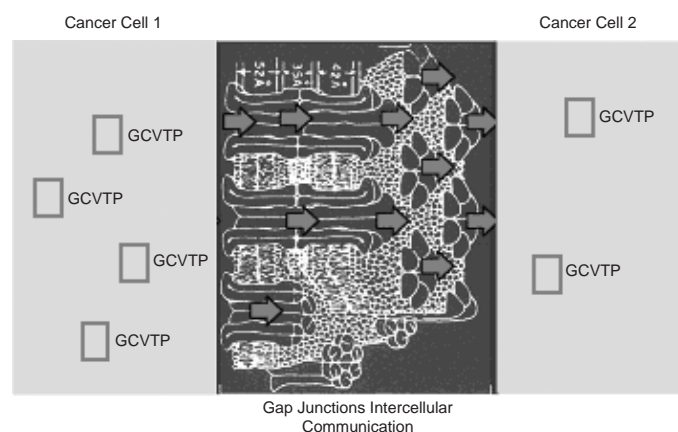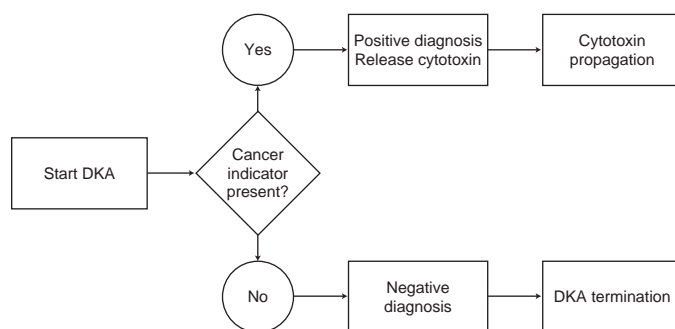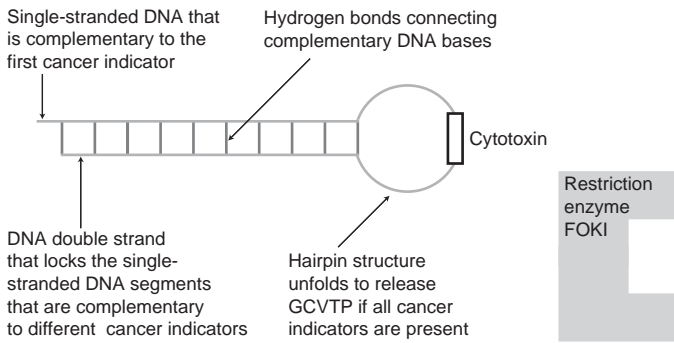
Figure 2
The *Bystander Effect*: cytotoxic chemicals carried by DKA are able to propagate from one cancer cell to another through the gap junctional intercellular communication (GJIC) channels.

Figure 4
DNA-based Killer Automaton: DKA has a structure similar to that of the molecular automaton model, proposed by Benenson *et al.* and shown in Figure 1. However, instead of DNA antisense that blocks cancer expression, DKA carries cytotoxic chemical, which is able to kill the cancer cell.
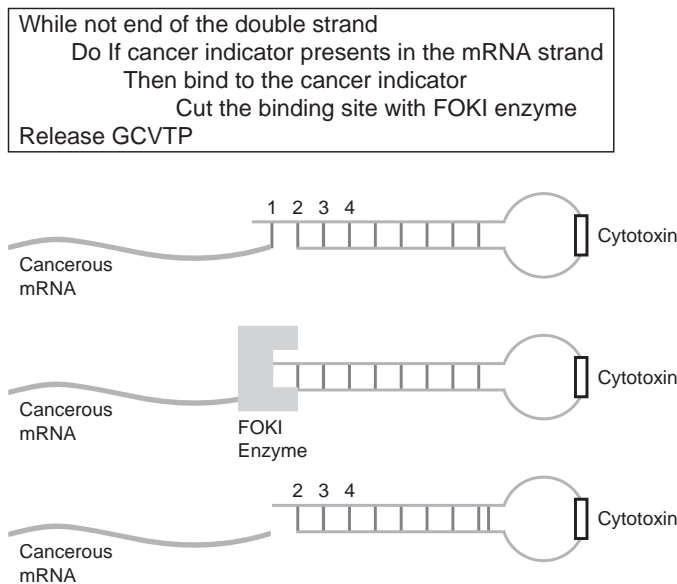
```
While not end of the double strand
    Do If cancer indicator presents in the mRNA strand
        Then bind to the cancer indicator
            Cut the binding site with FOKI enzyme
Release GCVTP
```


Figure 5
DKA Cancer Detection: the DKA cancer detector checks the presence of every cancer indicator in the mRNA strands. If all cancer indicators are detected, then cancer is diagnosed in the cell.

Due to the *bystander effect,* GCVTP released in one cancer cell is able propagate to neighboring cancer cells through homologous GJIC channels, eliminating those neighboring cells as well. GCVTP is not able to propagate to healthy cells due to the lack of GJIC channels, thus minimizing side effects. Additionally, if DKA enters a healthy cell accidentally, it does not harm the cell—without a complete set of cancer indicators, GCVTP is not unlocked.

The DKA model is only one part of a potential cure for cancer; a number of other challenges must be overcome as well. This paper focuses on the software simulation of the

DKA mechanisms, assuming that the other issues have been solved.

Tools and Methods

A software model has been established to simulate the DKA mechanism in an artificial multi-cell environment. The main tool used for this simulation is the Sun Java 2 Platform Standard Edition Software Development Kit (SDK), which serves as the compiler of Java simulation objects.

In this simulation, each component of DKA is constructed as a Java object and assigned specific chemical and physical properties, such as DNA/RNA base complementarities. There are three levels of objects in this simulation (Figure 6). The top level (Level 1) object is Experiment, which is an artificial multi-cell environment for the interactions between the two median level (Level 2) objects, DKA and Cell. Each Cell object contains multiple copies of two bottom level (Level 3) objects, GJIC and mRNA. GJIC objects are communication channels between cells that are able to transfer GCVTP. The mRNA objects serve as the identifiers of the host cell, and are used to decide whether the cell is cancerous or not.
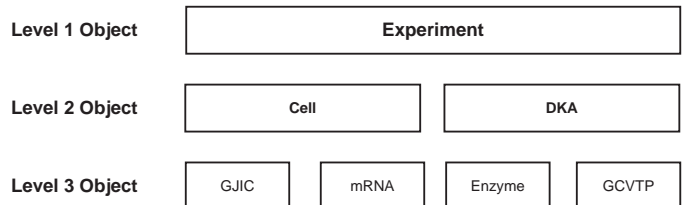

Figure 6
Architecture of the computer simulation model

Each DKA object also contains two Level 3 objects, Enzyme and GCVTP. Enzyme objects are used to cut binding sites after the cancer detector of a DKA object binds to a cancer indicator of an mRNA object. If the Cell object is cancerous, GCVTP objects are released to kill the host Cell object after cancer is diagnosed. Also, GCVTP objects are able to propagate from one cancer Cell object to another through the GJIC objects that connect these two Cell objects.

Creation of Cell Groups:
Two parameters, the number of cells, n, and cancer cell percentage, p, are used to generate cell groups. First, the program generates two mRNA objects, the cancerous and healthy mRNA. Then the program initializes n Cell objects and assigns cancerous mRNA to $p * n$ of them. The rest are

assigned healthy mRNA. Next, the program arranges the cancerous Cell objects so that they express cancer-cell localities, meaning that all cancer cells should be located close to each other since they are derived from the same origin. Each Cell object has four neighbors. If the neighbor cell is of the same type as the current cell, a GJIC object is created to facilitate communication between the two Cell objects. All cancerous Cell objects connect to each other through homologous GJIC channels, forming a cancerous cell line; therefore, every cancerous Cell object has at least one cancerous neighbor. Finally, the program mixes the healthy Cell objects and the cancerous cell line by randomly placing the healthy Cell objects around the cancerous cell line.

DKA Mechanisms:
The simulation of the DKA mechanisms takes place in three stages: distribution of DKA, cancer detection, and GCVTP propagation. For DKA distribution, the program takes the third parameter *d*, the number of DKA, and generates *d* DKA objects. It then randomly distributes them throughout all cells.

During chemical uptakes, cell volume increases. To avoid cell lysis, cell volume regulations are required to keep the cell volume stable (Lewis et al. 1990). These regulations usually involve ion channels that pump Na^+ and K^+ into/out of the cell when the cell volume varies (at the scale of ~10% of the isosmotic value). Unlike Na^+ and K^+, there are no ion channels that can pump DKA out of the cell; thus, in this simulation, a stricter restriction/assumption is imposed, such that a maximum number of 100,000 DKA (0.1% of the cell weight) can enter one cell without harming it. As a result, the program first randomly picks a cell, which can be healthy or cancerous. Then, it generates a random number, from 0 to 100,000, of DKA objects to enter the chosen cell. By repeating this process, the program distributes DKA to different cell Objects until all DKA have been distributed.

Cancer detection takes place after DKA distribution. After entering the Cell object, DKA objects use the detection algorithm to detect whether the host cell is cancerous or healthy. If the host cell tests positive for cancer, the GCVTP objects are released from the DKA objects. Otherwise, the DKA objects are terminated.

Finally, these GCVTP objects propagate from one cancer cell to another through the GJIC channels. GJIC channels allow chemicals and metabolites with molecular weight less than 1,000 Da to diffuse from one cell to another; thus GCVTP, which has a molecular weight of 496 Da, is able to diffuse through GJIC channels. However, due to the degra-

dation of the DKA structure, not all GCVTP is released in cancer cells as expected. In addition, due to the chemical degradation (Cornors et al. 1986) of GCVTP (or other cytotoxic materials), not all GCVTP released in one cancer cell is able to propagate to nearby cancer cells. Consequently, the diffusion of GCVTP is not uniform, and there is an equilibrium condition at which all GCVTP objects stabilize and are no longer able to propagate. To take these two kinds of degradation into consideration, the threshold for disrupting the equilibrium condition is defined as a difference of 10,000 DKA (10% of the maximum DKA that is allowed to enter one cell) between two neighboring cancer cells. Hence, GCVTP continues to propagate until all cancer cells have reached the equilibrium condition. After the equilibrium condition has been reached, the program kills the cells that contain GCVTP objects.

Experiments and Results

Two different simulations demonstrate how DKA could destroy cancer malignancies with minimal side effects, and predict the efficacy of DKA. The first is the single-cell/single-DKA simulation, which demonstrates in detail how a DKA detects cancer and releases GCVTP if cancer is diagnosed in a cell. The second is the multiple-cell/multiple-DKA simulation, which demonstrates the efficacy of DKA with various cancer cell percentages and units of DKA injected.

Single-Cell/Single-DKA Simulation
In this simulation, a DKA object is constructed to detect the cancer indicators of the p53 gene, which is very frequently mutated in small-cell lung cancer (SCLC). This gene contains the cancer indicators CCUUUAU, AAGUAAA, CCAAAAG, and CGACGAA (Takahashi et al. 1991). To detect SCLC, this DKA must include a cancer detector containing DNA segments GGAAATA, TTCATTT, GGTTTTC, and GCTGCTT.

First, the DKA object is initialized in a cancer cell object that expresses the SCLC p53 gene mutation. As Figure 7a shows, the DKA cancer detector checks and cuts each cancer indicator until the whole mRNA object has been checked. Then, after verifying that the cell object is cancerous, DKA releases GCVTP to kill the cell. Next, the same DKA object is initialized in a healthy cell, which contains the mRNA object CCUUUAU-AAUUAAA-CCAAAAG-CGACGAA. As Figure 7b shows, since the first segment CCUUUAU is complementary to the first segment of the cancer detector, DKA cuts the first segment and releases

A.
```
Cell has been initialized!
This cell contains mRNA CCUUUAUAAGUAAACCAAAACCGACGAA.

DKA has been initialized!  This DKA has the following structure:
1. The cancer detector has the follow structure:
     lock: TTCATTTGGTTTTCGCTGCTT
     probe: GGAAATA
2. At the end of the detector there is a GCVTP attached
3. Enzymes are also part of DKA

DKA have been injected into the target cell.
Binding successful!  Bond formed between DNA probe GGAAATA and mRNA
sequence CCUUUAU.
Strand cut! Cut from mRNA: CCUUUAU.  Cut from DKA: GGAAATA.

Binding successful!  Bond formed between DNA probe TTCATTT and mRNA
sequence AAGUAAA.
Strand cut! Cut from mRNA: AAGUAAA.  Cut from DKA: TTCATTT.

Binding successful!  Bond formed between DNA probe GGTTTTC and mRNA
sequence CCAAAAG.
Strand cut! Cut from mRNA: CCAAAAG.  Cut from DKA: GGTTTTC.

Binding successful!  Bond formed between DNA probe GCTGCTT and mRNA
sequence CGACGAA.
Strand cut! Cut from mRNA: CGACGAA.  Cut from DKA: GCTGCTT.
The whole mRNA strand has been checked and cut!

GCVTP is released and Cell will be killed!
```

B.
```
Cell has been initialized!
This cell contains mRNA CCUUUAUAAUUAAACCAAAAGCGACGAA.

DKA has been initialized!  This DKA has the following structure:
1. The cancer detector has the follow structure:
     lock: TTCATTTGGTTTTCGCTGCTT
     probe: GGAAATA
2. At the end of the detector there is a GCVTP attached
3. Enzymes are also part of DKA

DKA have been injected into the target cell.
Binding successful!  Bond formed between DNA probe GGAAATA and mRNA
sequence CCUUUAU.
Strand cut! Cut from mRNA: CCUUUAU.  Cut from DKA: GGAAATA.

Binding failed!  This is not a cancerous cell!
```

Figure 7
Single-Cell/Single-DKA Simulation result: (A) DKA checks the presence of all cancer indicators and confirms that the cell is cancerous. (B) Since not all cancer indicators are detected, DKA terminates and concludes that the cell is not cancerous.

the second segment AAGUAAA. However, the second mRNA segment is not complementary to the second segment of the cancer detector, which implies that this cell is not cancerous. DKA terminates without releasing GCVTP.

Multiple-Cell/Multiple-DKA Simulation
In this simulation, three cell groups were created: 1,000 cells in which 10% were cancerous, 1,000 cells in which 50% were cancerous, and 1,000 cells in which 100% were cancerous. In each of these cases, 21 different experiments were conducted by adjusting the amount of DKA used. For each experiment, both the percentage of cancer cells initially entered by DKA and the percentage of cancer cells killed by GCVTP after toxicity propagation were recorded. To make sure that the results were reliable, each experiment was repeated 100 times, and the average value of these samples was taken as the final result of each experiment.

Figure 8 compares the percentage of cancer cells killed with the percentage of cancer cells initially entered by DKA. For 1,000 cells in which 10% are cancerous, the cancer cell killing becomes reliable (>95%) if DKA have entered more than 18.9% of cancer cells initially. For 1,000 cells with 50% cancer cells, the cancer cell killing becomes reliable if DKA have entered more than 8.3% of cancer cells initially. For 1,000 cells with 100% cancer cells, the cancer cell killing becomes reliable if DKA have entered more than 2.05% of cancer cells initially. This confirms that DKA does not have to enter all cancer cells initially to destroy cancer malignancies; as long as they can enter a certain threshold percentage of all cancer cells, the toxicity they carry can propagate to all cancer cells, resulting in complete tumor regression. In addition, this data demonstrates that a higher cancer cell percentage results in a lower threshold percentage. 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 GCVTP. In the 50% cancer cell case, the threshold percentage for complete tumor regression is 10.5%, which agrees with the data obtained from suicide gene therapy experiments with 50%-50% cancerous to healthy cell culture (Culver etc. 1992).

Figure 9 compares the percentage of cancer cells killed with the amount of DKA used. For 1,000 cells with 10% cancer cells, the cancer cell killing becomes reliable (>95%) after the number of DKA has reached 5,000,000. For 1,000 cells with 50% cancer cells, the cancer cell killing becomes reliable after the number of DKA has reached 3,000,000. For 1,000 cells with 100% cancer cells, the cancer cell killing becomes reliable after the number of DKA has reached 1,000,000. This shows that the percentage of cancer cells killed is directly related to the number of DKA, and the percentage of cells that are cancerous.
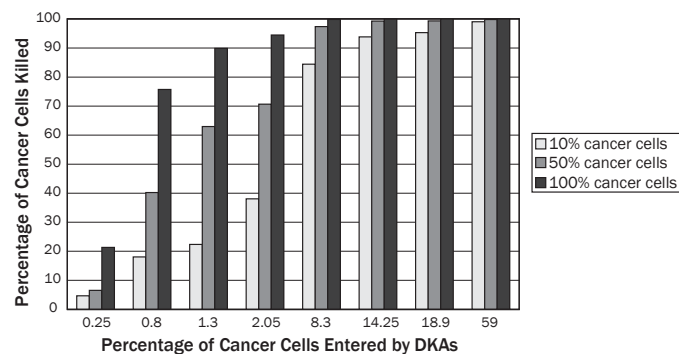

Figure 8
Multiple-Cell/Multiple-DKA Simulation Result Part I: The relationship between the percentage of cancer cells initially entered by DKA and the percentage of cancer cells killed.
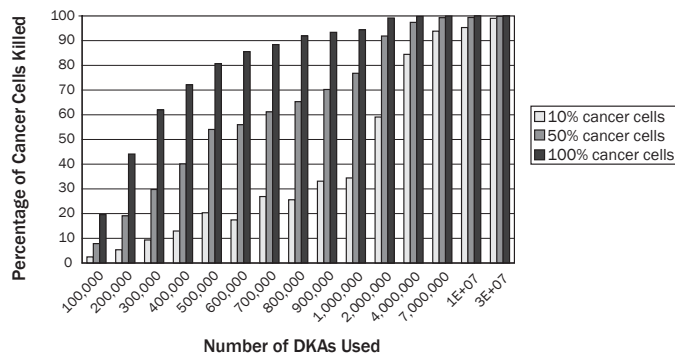
Figure 9

Multiple-Cell/Multiple-DKA Simulation Result Part II: The relationship between the amount of DKA used (dose) and the percentage of cancer cells killed (efficacy).

Figure 10

Multiple-Cell/Multiple-DKA Simulation Result Part III: The relationship between the amount of DKA used (dose) and the percentage of cancer cells initially entered by DKA.

Figure 10 compares the percentage of cancer cells initially entered by DKA with the number of DKA used. This demonstrates that the percentage of cancer cells initially entered by DKA depends on the number of DKA injected to the cell group, but is independent of the percentage of cells that are cancerous. This is reasonable because the percentage of cells that are cancerous determines the density of homologous GJIC channels and, consequently, how well GCVTP can propagate among cancer cells. Thus, it does not affect the spread of DKA.

To identify the relationships between the efficacy of DKA, the cancer cell percentage, and the amount of DKA injected into the cell group, simulation results have been reorganized in Figure 11 with estimation trend lines. As shown in Figure 11A, for a fixed cancer cell percentage, the efficacy of DKA linearly depends on the number of DKA injected into the cell group before saturation. After reaching saturation, increasing the number of DKA has little effect on the killing efficacy. For instance, the trend line matches the dose-response relationship of the 100% cancer cell case before the dose reaches 500,000 DKA, which results in 80% tumor regression. However, after the dose reaches 500,000 DKA, the change of cancer cell killing efficacy slows down. On the other hand, for a fixed number of DKA used, as shown in Figure 11B, the efficacy of the DKA method also 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), which is described by Equation 1.

$$E = Constant * D * N \qquad (1)$$

There are several scatters that deviate from the trend lines in the figures. These scatters may be the result of the randomness of the software model. As indicated earlier, DKA
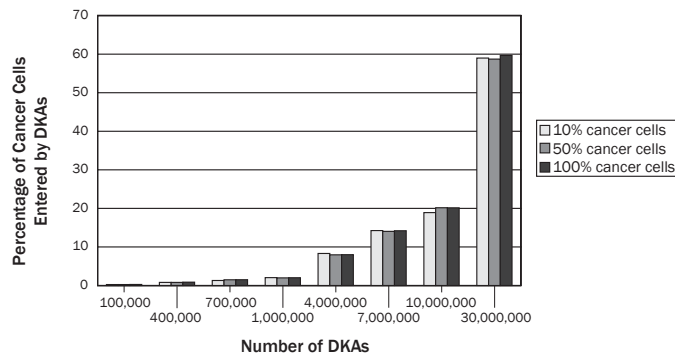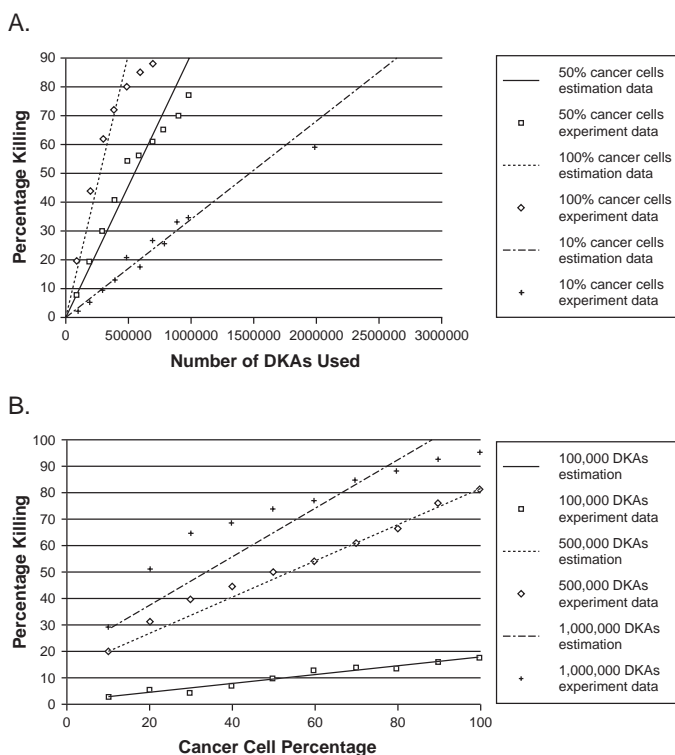
A.

B.

Figure 11

The relationships between cancer cell percentage, the amount of DKA used (dose), and the percentage of cancer cells killed (efficacy).

are distributed randomly through all cells to keep the model simple. Consequently, the DKA efficacy varies according to this distribution.

Conclusion and Future Work

This paper proposes the DNA-based Killer Automaton, an innovative intelligent nanomedicine that has the potential to completely destroy cancer malignancies in one treatment

with minimal side effects. For this DKA model to work *in vivo,* four major research challenges have been identified: (1) A method to deliver DKA to mammalian cells is not yet available. Possible solutions to this problem include Retrovirus vectors, Adeno-Associated Virus (AAV) vectors, and gene guns. (2) The restriction enzyme FOKI is not usually present in mammalian cells. If it is introduced into a mammalian cell, it might cut random mRNA strands in a healthy cell, therefore preventing DKA from working *in vivo* at this stage. One possible solution is to deliver this enzyme to cancer cells in DNA form with mammalian expression promoter attached (Takebe et al. 1988). (3) There is no literature about how to attach GCVTP to a DNA strand, although it could theoretically be done through chemical synthesis or cross-linking. (4) A reliable method of releasing GCVTP from DKA without losing its cytotoxicity is needed. One possible solution is to identify an enzyme that can separate DNA and GCVTP without changing the chemical structure of GCVTP.

This research demonstrates that the DKA has the potential to provide a promising approach to cure genetic mutation diseases, such as cancer. If successfully applied *in vivo,* DKA could also be used to cure other genetic mutation diseases. The next stage of this research is to conduct an *in vitro* experiment on this DKA model in a lab environment, so that its efficacy *in vitro* can be evaluated and its *in vivo* feasibilities can be predicted.

Acknowledgements

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