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Systems Biology Approach of Understanding the Effect of *Raphanus sativus* Extract on the Pathogenesis of Chronic Myeloid Leukemia, Breast and Colon Cancer

Jan Marie Claire J. Edra[1], Kathleen Dane T. Talag[1], Ma. Luisa D. Enriquez[2,3] and Angelyn R. Lao[1,3*]

[1]Department of Mathematics, De La Salle University
[2]Department of Physics, De La Salle University
[3]Mathematical and Statistical Modeling Center, CENSER, De La Salle University
*Corresponding author: angelyn.lao@dlsu.edu.ph

Abstract: Chronic myeloid leukemia (CML), breast, and colon cancer are among the most prevalent types of cancer in the Philippines. In most of the cancer cases reported annually, cancer is only detected when it is on its advance stage due to the lack of early detection and limited options of targeted therapies. There are studies that show different effects of *Raphanus sativus* (radish) extract on different cancer diseases. However, it is still unknown why it has different effects on different cancer diseases. Therefore, understanding disease dynamics would help in improving prognosis and diagnosis. In this study, we propose a mathematical model that would explain the effect of *Raphanus sativus* extract on the pathogenesis of CML, breast and colon cancer. Since p53 is one of the most extensively studied gene in the field of cancer research and Cyclin D1-CDK complex is one of the more frequently altered cell cycle regulators in cancers, these genes are the focus for the basis of the effectiveness of the drug. Based on our simulations, we have showed that a 99.99%, 99.68%, and 98.96% decrease in the steady-state concentration of p53 are observed in breast cancer, colon cancer, and CML, respectively, after the drug was inserted in the model. Moreover, Cyclin D1-CDK complex's steady-state concentration is decreased by 99.91% in breast cancer, 99.89% in colon cancer, and 98.50% in CML. Hence, the model suggests that *Raphanus sativus* extract is highly effective on breast cancer followed by colon cancer and CML.

Key Words: Mathematical modeling; *Raphanus sativus*; Chronic myeloid leukemia; breast cancer; colon cancer

1. INTRODUCTION

Cancer is the third leading cause of death in the Philippines, and chronic myeloid leukemia (CML), breast, and colon cancer are three of the most prevalent types (Ngelangel & Wang, 2002). Annually, there are about 900, 12800, and 8000 cases of CML, breast, and colon cancer, respectively. In most of these cases, cancer is only detected on its advance stage due to the lack of early detection and limited options of targeted therapies (Au et al., 2009; Buban, 2013; Tubeza, 2012).

Raphanus sativus (radish) is a root vegetable of the Brassicaceae family (Jeff Conner, Ian Dworkin, Shin-Han Shiu, Yongli Xiao, & Cindi Mills, 2009). Some literature investigated for this natural product's cancer preventive effects. Kim et. al. (W. K. Kim et al., 2011) studied on the effects of ethanol

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extract of aerial parts of *Raphanus sativus* L. leaf on breast cancer cell proliferation and gene expression and was able to show that it suppresses the EGFR-AKT pathway which includes RAS. On the other hand, analysis of the inhibitory effects of *Raphanus sativus* root extract in human cancer is the focus of the study by Beevi et. al. (Beevi, Mangamoori, Subathra, & Edula, 2010). They discovered that the hexane extract of *Raphanus sativus* root is having interactions with p53 and may also have with Bcl(2) family of genes, which includes Bad. In an article by Cragg and Newman (G. M. Cragg & D. J. Newman, n.d.) they stated that olomucine, isolated from the cotyledons of radish, is shown to inhibit cyclin dependent kinases (CDKs).

In a study by Roleda and Tan (2013), the cytotoxic and genotoxic effects of *Raphanus sativus* extract were tested on four cell lines, namely, breast cancer, colon cancer, leukemia and a normal cell. On the cytotoxicity test, the different cell lines were cultured and treated in a 96-well plate with *Raphanus sativus* extract and were added with Presto blue cell viability reagent, a based fluorescent dye used to measure cell cytotoxicity. For genotoxicity test, micronucleus test was used. All cell lines were experimented through in vitro. Their results showed that radish extract was most cytotoxic and genotoxic on breast cancer cell line. It was also cytotoxic on colon cancer cell line but not on leukemia. The least cytotoxic and genotoxic effect of the extract were observed on the normal cell line.

For understanding the complexity of disease dynamics, analyzing observed patterns focused on mechanistic hypothesis, and testing feasible control measures, mathematical modeling is a useful and helpful instrument. It plays an important role in interpreting complex systems in biology (Tsygvintsev, Simeone, & Kirschner, n.d.).

With cancer being one of the deadliest and complex diseases, we want to determine the genes that are found in signaling pathways which could be significant in prognosis and diagnosis. Scientists may use this study to know which genes to focus on for drug discovery. For other studies of the same interest, this study may be a source of comparison in terms of the genes that we found and the results that we obtained.

2. MATERIALS & METHODOLOGY

We now discuss the methods employed in the construction of the proposed mathematical model for CML, breast, and colon cancer.

Before establishing the mathematical model, we formed three signaling pathways (see Figure 1) for each of the three diseases. First, we looked for complex pathways associated to the three cancers. Second, we identified the genes which are common to all of the three diseases and which are unique to each of them. Last, we added the drug, *Raphanus sativus* extract, as developed in the study by Roleda and Tan (2013) where they tested the effect of this natural product on leukemia, breast and colon cancer. Considering all of these, we came up with our proposed signaling pathways for CML, breast, and colon cancer.

After constructing the signaling pathways, we created the mathematical model (see Table 1). Each components of the signaling pathways were encoded to ordinary differential equations by applying some concepts in enzyme kinetics. We then calibrated the parameter values in order to satisfy the effect of the drug observed in the study by Roleda and Tan (2013). MATLAB's ode solver, ode15s, was used to generate the solutions for the system of ordinary differential equations and to produce simulations which will explain the behavior of these equations.

2.1 Signaling Pathways

The activities of a group of molecules working together to control cell functions, such as cell division or cell death, are described in a signaling pathway (National Cancer Institute, n.d.). Malfunctioning components of signaling pathways can lead to cancer (Berg, Tymoczko, & Stryer, 2002). Hence, closer examination on these signaling pathways will greatly contribute in analyzing the characteristics of this complex disease.

Our proposed pathways are products of a series of investigation from the literature. Beginning with complex pathways of CML, breast cancer, and colon cancer, the common genes for three cancers were identified. The resulting signaling pathways for CML, breast cancer, and colon cancer are shown in Figures 1A, 1B, and 1C, respectively.

After establishing the signaling pathways of the three diseases, the drug was inserted. The drug

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inhibited RAS, p53, and Cyclin D1-CDK complex for all the three diseases. However, there were some genes, not associated to all the three cancers, which are inhibited by the drug as well. For CML and breast cancer, the drug also inhibited BCR-ABL. But, Bad is an additional gene in CML which is repressed by the drug. In colon cancer, the other genes inhibited by the drug are MMR, MSI, DCC, and Bad.

2.2 Model Equations

We now establish a mathematical model that represents the interaction of the genes in the three signaling pathways. We do this by generating three system of ordinary differential equations, one for each of the three types of cancer. For CML, these are the equations shown in Table 1A, 1B, 1C, and 1E. For breast cancer, equations included in Table 1A, 1B, and 1E make up the system. In colon cancer, we have the equations presented in Table 1A, 1C, 1D, and 1E. Whereas, the equation for the drug contains a negative deactivation rate denoted by $-\delta_Z \cdot Z$ since its concentration decreases when it is distributed to some of the genes.

3. SIMULATION REULTS

In this paper, we will only demonstrate the behavior of the cancer genes that are found in the pathway breast cancer (Figure 2). Simulations of the model equations were done using MATLAB. We used ode solver, ode15s, in order to simulate the systems of ordinary differential equations that represent the pathways of the three cancers. All the genes have certain behaviors which are observable for the three cancers. With the presence of drug, steady-state concentration of these genes are expected to be increased or decreased depending on their behavior in the absence of the drug. Since p53 is one of the most extensively studied gene in the field of cancer research (Adami, 2008) and Cyclin D1-CDK complex is one of the more frequently altered cell cycle regulators in cancers (J. K. Kim & Diehl, 2009), these genes are the focus for the basis of the effectiveness of the drug. In addition, it is also interesting to know the change in steady-state concentrations of the other genes to be able to recognize how the drug affected these genes.

4. CONCLUSIONS

With CML, breast, and colon cancer as some of the most predominant types of cancer in the Philippines, understanding the disease dynamics is important for effective prognosis and diagnosis. Through mathematical modeling, more comprehensive knowledge about this disease can be achieved. In this paper, we have proposed a mathematical model which would explain the behavior of the components of the signaling pathways of CML, breast cancer, and colon cancer in the absence and presence of a drug, *Raphanus sativus* extract.

Based on the literature and study by Roleda and Tan (2013), we presented signaling pathways for each of the three diseases. To be able to mimic the effect of *Raphanus sativus* extract into the three cancers as observed in the study by Roleda and Tan (2013), we hypothesize that the drug inserted in CML signaling pathway could inhibit BCR-ABL, RAS, Bad, and p53. For breast cancer signaling pathway, the drug could inhibit BCR-ABL, RAS, and p53. In colon cancer, however, there were many genes in which the drug could have a direct effect. The genes inhibited are RAS, DCC, MMR, MSI, Cyclin D1-CDK complex, Bad, and p53.

We showed that, in the absence of drug, all the genes' concentration in CML and breast cancer are upregulating. In colon cancer, all other genes' concentration except for DCC and MMR are also increasing. All of our results are consistent with what have been shown in previous studies. After the drug is inserted in the model, all the genes' steady-state concentration in CML and breast cancer decreased. This was also the case for all other genes' steady-state concentration except for DCC and MMR in colon cancer. Based on our model, *Raphanus sativus* extract is highly effective in breast cancer followed by colon cancer and CML on the basis of p53's and Cyclin D1-CDK complex's percent decrease in steady-state concentration before and after the drug is inserted in the model, and these results are similar to what have been observed in the study by Roleda and Tan (2013). Our outcome shows that there was a 99.99%, 99.68%, and 98.96% decrease of p53 in breast cancer, colon cancer, and CML, respectively. Cyclin D1-CDK complex, on the other hand, is decreased by 99.91% in breast cancer, 99.89% in colon cancer, and 98.50% in CML.

For future studies, it is recommended that our model be validated in the laboratory. We also proposed that the pathways be extended for further investigation.

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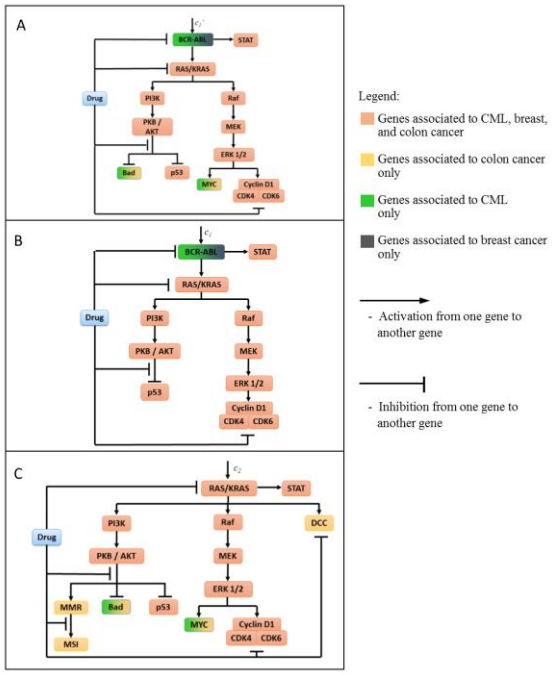

Figure 1. Signaling pathways of CML (A), breast cancer (B), and colon cancer (C) in the presence of the drug, *Raphanus sativus*.

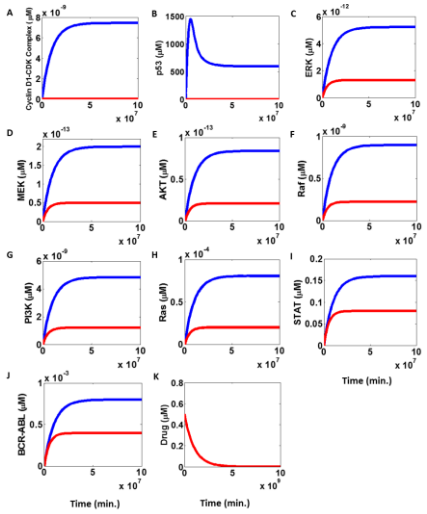

Figure 2: Simulation results for the breast cancer mathematical model with and without the presence of a drug.

A	$\frac{dS}{dt} = \mu_S \cdot Br - \delta_S \cdot S$	(1)
	$\frac{dP}{dt} = \mu_P \cdot R - \delta_P \cdot P$	(2)
	$\frac{dA}{dt} = \mu_A \cdot \frac{P}{P + \kappa_A} - \delta_A \cdot A$	(3)
	$\frac{dF}{dt} = \mu_F - \delta_F \cdot F \cdot \frac{A}{A + \kappa_F} - \delta_{FZ} \cdot F \cdot \frac{Z}{Z + \kappa_F}$	(4)
	$\frac{dRf}{dt} = \mu_{Rf} \cdot R - \delta_{Rf} \cdot Rf$	(5)
	$\frac{dM}{dt} = \mu_M \cdot Rf - \delta_M \cdot M$	(6)
	$\frac{dE}{dt} = \mu_E \cdot M - \delta_E \cdot E$	(7)
	$\frac{dD}{dt} = \mu_D \cdot E - \delta_D \cdot D + \mu_{DZ} - \delta_{DZ} \cdot D \cdot \frac{Z}{Z + \kappa_D}$	(8)
B	$\frac{dBr}{dt} = c_1 - \delta_{Br} \cdot Br - \delta_{BrZ} \cdot Br \cdot \frac{Z}{Z + \kappa_{Br}}$	(9)
	$\frac{dR}{dt} = \mu_R \cdot Br - \delta_R \cdot R + \mu_{RZ} - \delta_{RZ} \cdot R \cdot \frac{Z}{Z + \kappa_R}$	(10)
C	$\frac{dMC}{dt} = \mu_{MC} \cdot E - \delta_{MC} \cdot MC$	(11)
	$\frac{dB}{dt} = \mu_B - \delta_B \cdot B \cdot \frac{A}{A + \kappa_B} - \delta_{BZ} \cdot B \cdot \frac{Z}{Z + \kappa_B}$	(12)
D	$\frac{dR}{dt} = c_2 - \delta_R \cdot R - \delta_{RZ} \cdot R \cdot \frac{Z}{Z + \kappa_R}$	(13)
	$\frac{dDC}{dt} = \mu_{DC} \cdot E - \delta_{DC} \cdot DC + \delta_{DCZ} \cdot DC \cdot \frac{Z}{Z + \kappa_F}$	(14)
	$\frac{dMS}{dt} = \mu_{MS} \cdot MR - \delta_{MS} \cdot MS + \mu_{MSZ} - \delta_{MSZ} \cdot MS \cdot \frac{Z}{Z + \kappa_{MS}}$	(15)
	$\frac{dMR}{dt} = \mu_{MR} \cdot A - \delta_{MR} \cdot MR + \mu_{MRZ} - \delta_{MRZ} \cdot MR \cdot \frac{Z}{Z + \kappa_{MR}}$	(16)
E	$\frac{dZ}{dt} = -\delta_Z \cdot Z$	(17)

Table 1: Model equations associated to all three cancers (A), to CML and breast cancer (B), to CML and colon cancer only (C), to colon cancer only (D) and to the drug (E).