

ODE Analysis of a Cancer Pathway

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1 Introduction

Despite decades of research, cancer still remains a leading cause of death among the human population. In 2004, the World Health Organization released statistics which showed that 12.5% of the deaths worldwide are a result of cancer related malignant neoplasms [1]. Sadly, this percentage does not even account for deaths related to lung cancer, stomach cancer, and other cancers of the body. Thus, there remains a critical need for effective treatments against these tragic illnesses.

Unfortunately, the science behind cancers is highly complex, which directly contributes to the difficulty of finding effective treatments. Recent research has focused on the study of biochemical pathways and their relation to uncontrollable cell proliferation. By pooling knowledge about such pathways, highly detailed maps have been constructed which illustrate both the types of mutations that can occur, and the effects these mutations have on the overall pathway. By identifying the key mutations and interactions which lead to cell proliferation, it is hoped that treatment efficacy can be drastically improved. The difficulty with this method lies in the complexity of the biochemical pathways. A single pathway, for example, may experience tens of mutations which are either directly, or indirectly, involved in hundreds of interactions. Thus, there is no definite way to *a priori* identify suitable targets for a drug treatment.

Mathematically modelling these biochemical pathways, however, does offer hope for this strategy. In particular, a mathematical model can take into consideration the numerous mutations and interactions which occur while allowing for a systematic study of the effects that a specific drug might have on the system. By accurately modelling the pathway, the key interactions can be identified from a theoretical point of view, which may suggest possible treatments to explore in the real world.

In this report, a mathematical study identical to the one described above is performed on a simplified version of the basal cell carcinoma pathway. More specifically, the full biochemical pathway is reduced by only considering its primary interactions and processes. From this simplified pathway, a system of ordinary differential equations is derived which describe the concentration of key proteins and ligands. Furthermore, the effects of two hypothetical treatments, each of which target a different interaction, are investigated. Section 2 describes, in detail, the basal cell carcinoma pathway while Section 3 is devoted to the model and the results that are obtained from it. The report then concludes with a brief summary and discussion.

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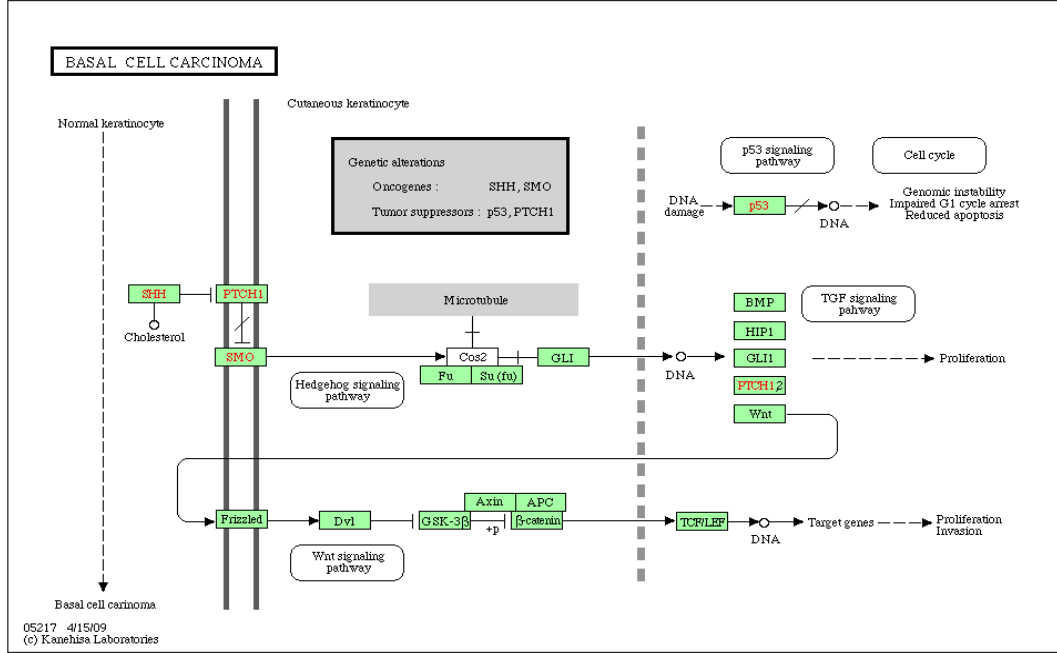


Figure 1: The Basal cell carcinoma pathway. Image from [6].

2 The basal cell carcinoma pathway

A first step to creating an effective cancer treatment is understanding underlying biological processes and key interactions in a cell that lead to uncontrollable cell division and therefore cancer. One approach to understanding reactions taking place in a cancerous cell is to consider so-called signal transduction pathways or signaling pathways. In general, the term signaling pathway refers to any ordered sequences of biochemical reactions inside the cell, which are carried out by enzymes and activated by second messengers. Most signaling pathways involve the binding of extracellular signaling molecules (or ligands) to cell-surface receptors that face outward from the plasma membrane and trigger events inside the cell. The usual cellular responses to the activation of a signalling pathway include activation of genes, alterations in metabolism, the continued proliferation, and death of the cell. If mutations occur in a cell they can cause an uncontrolled activation of some pathways leading to fast proliferation and cancer tissue formation. Different signaling pathways interact with each other thus creating a complex net of processes in a cell that can be represented by a biochemical pathway map. The Kyoto Encyclopedia of Genes and Genomes (KEGG) database [6], for example, contains a collection of pathway maps representing the most up-to-date knowledge on the molecular interaction and reaction networks for cellular processes including those in pathogenic cells. Most of the pathway maps for the well-known human cancers are very complicated and understanding them doesn't seem to be a feasible task for non-biologists. One of the simplest maps is the map for a widespread skin cancer called basal cell carcinoma (see Figure 1) on which we will concentrate our attention.

From Figure 1 we can see four signaling pathways engaged in basal cell carcinoma development.

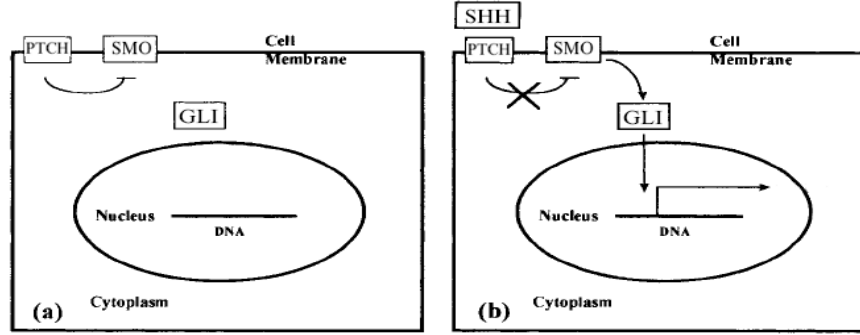


Figure 2: Hedgehog signaling pathway.

The first pathway, called the *p53* signaling pathway, leads to a reduced apoptosis which means cell death, hence no further cancer growth. The other three — Hedgehog, TGF, and Wnt pathways — lead to proliferation of cancerous cells and therefore are responsible for the development of cancer. Many pathways that play an essential role during the embryonic development are switched off later in life, during adulthood. Aberrant activation of these pathways in adult tissue is often oncogenic. It is known (see e.g. [4]) that the Hedgehog signalling pathway is important in embryological development and is highly conserved through evolution. Activation of this pathway has been implicated in the development of various cancers including basal cell carcinoma, which was proved to have the closest association with hedgehog signaling [3]. Since it is believed that inhibiting the HH (Hedgehog) pathway can provide an efficient therapy for a wide range of malignancies [2] including the one under consideration, we chose to concentrate our attention on this particular pathway and its signaling mechanisms.

Returning to the diagram on Figure 1 we will consider the part of it which is engaged in the HH pathway. From the diagram one can see two transmembrane proteins built in cell membrane — PTCH (Patched), or more specifically, the PTCH1 homologue, and SMO (Smoothened) — the first of which serves as a receptor for the secreted protein SHH (Sonic Hedgehog). In the absence of SHH, PTCH1 inhibits the HH pathway by repressing the activity of SMO. Binding of the SHH ligand to PTCH1 relieves its inhibition of SMO, which in turn triggers the release of the GLI protein family transcription factors from the protein complex (Cos-2, SU and SUFU on Figure 1). The activation of GLI protein drives an overexpression of downstream genes in the nucleus and thus proliferation and differentiation takes place.

A large number of different factors can affect the work of HH signaling pathway. Studies have shown [2, 3] that deregulation of the HH pathway in basal cell carcinoma occurs by mutation or altered activity of one or more of the members. Proteins expressed by mutated genes or altered by external factors are shown in red on the Figure 1. While the Hedgehog signaling results in overexpression of PTCH, SMO and GLI genes, it should also be noted that it activates the Wnt signaling pathway which eventually leads to expression of SHH gene [5]. Expression of both PTCH1 and SHH creates a positive feedback loop in a cancer development through HH signaling. A simplified scheme of the Hedgehog signaling is shown on Figure 2.

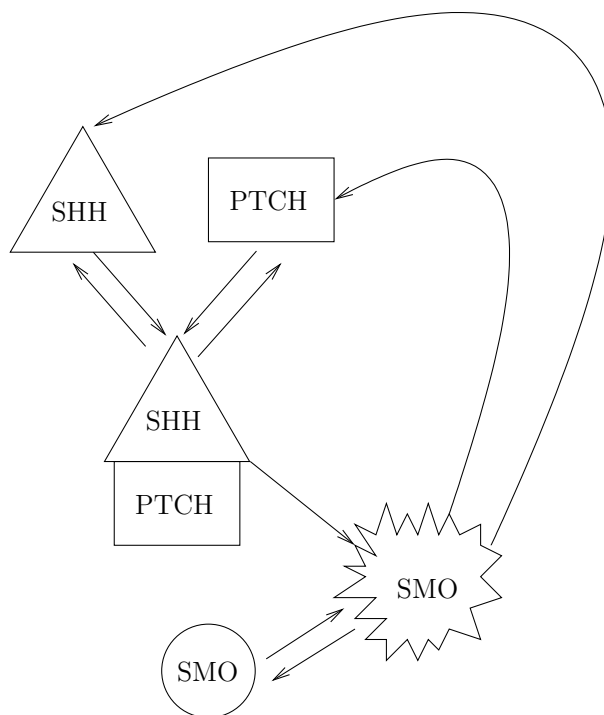


Figure 3: The simplified pathway created to model cancer growth in a skin cell.

3 Model

Looking at the large biological pathway diagram referenced previously (Figure 1), the complexities in even a relatively simple cancer pathway are clear and thus a thoroughly accurate model is difficult to generate, especially considering the lack of modelling literature found on this topic.

Hence, after discussion with the biological research department at the University of Calgary, we decided upon a way to create a simplified pathway that could be modelled. We noticed that at the beginning of the pathway, the mutated SHH combines with a mutated PTCH1 creating a complex which activates SMO. The creation of this activated SMO continued on through the pathway to stimulate cancer proliferation through a multitude of intermediate steps. Therefore, we decided that by modelling the concentration of this activated SMO we could have an estimate on cancer production because of this direct cancer growth relation. The simplified pathway is presented in Figure 3. The oversimplification step is the positive feedback loop where the activated SMO produces new SHH and PTCH1 as this in actuality is an indirect reaction. In our model we consider five concentrations, x_1 for SHH, x_2 for PTCH1, x_3 for the SSH PTCH1 binding complex, x_4 for the inactive SMO, and $\tilde{x}_4$ for the activated SMO. A simple mass action law approach was used whereby reactants in a synthesis equation are combined as products in the differential equation indicating that no reaction is possible when either reactant is absent. The reactions from Figure 3

can be simplified to



where SPc is the SHH-PTCH1 complex and aSMO is the activated SMO. The SHH is lost by the synthesis of SPc with PTCH1 and then produced again by the dissociation of the SPc back into its constituents along with the production of new species from the aSMO. This is the same reaction as with the PTCH1. The complex is created by the synthesis of the SHH and PTCH reactants but naturally dissociates back to the individual compounds and the SMO concentration is increased as the aSMO loses its energy but is decreased by the activation with the SHH-PTCH1 complex. These are all modelled with ordinary differential equations in (2),

$$\dot{x}_1 = -ax_1x_2 + bx_3 + c\tilde{x}_4 \quad (2a)$$

$$\dot{x}_2 = -ax_1x_2 + bx_3 + c\tilde{x}_4 \quad (2b)$$

$$\dot{x}_3 = -bx_3 + ax_1x_2 \quad (2c)$$

$$\dot{x}_4 = c\tilde{x}_4 - bx_3x_4 \quad (2d)$$

$$\dot{\tilde{x}}_4 = bx_3x_4 - c\tilde{x}_4 \quad (2e)$$

where a , b , and c represent the three key reaction rates. For generality, each reaction would have a different rate constant but conservation of mass can simplify them down to three (possibly) unique rates. For instance, the SHH-PTCH1 complex forms with equal parts of each constituent so the rate of synthesis of SPc must be the same as the loss rate of each SHH and PTCH1 respectively. This is also similar for the complex dissociation. Since the activated SMO is synthesized from a one to one reaction with the complex and inactive SMO then the same reasoning is used to justify the third rate constant. Without proper empirical data, a robust justification for rate constant values is impossible, however, we approximated them with some physical justification. The rate constant a was set to 1 without loss of generality with everything else being evaluated in reference to that. The rate constant b was then set to $1/2$. This was justified under a conservation of mass ideology whereby if two reactants form a single complex with rate constant a then in the reverse reaction, the rate at which the two original reactants are reproduced must be half of the original rate. This is similar to current branching in electrical circuits. The rate constant c which relates to the production of activated SMO was left as an adjustable parameter.

3.1 Cancer Proliferation and Drugs

As mentioned, the complex pathway diagram shows that after the SMO is activated it eventually ends up in a step with cancer proliferation. Therefore, the concentration of the aSMO is directly proportional the cancer production in the basal cell for any time t . The rate of cancer development is then going to be,

$$C = \int_0^t c\tilde{x}_4(s)ds. \quad (3)$$

By introducing a drug into the pathway, the goal is to limit the cancer proliferation. While this can be done in a various number of ways from a physical perspective, the only qualitative change would

be a decrease in the rate of production of cancer. Therefore, adding drugs into the system can be modelled as varying the rate constant c attached to the production of activated SMO. For model results, we varied the rate constant from 0 (the perfect drug) to 1 (no drug acting) and monitored effects. We also considered a drug that affected the synthesis of the SHH and PTCH1 complex.

3.2 Results

Due to the nonlinearities in the governing equations (2), an analytical solution could not be found. Furthermore, a linear stability analysis of the equations is nontrivial because the equations which govern the steady state are not independent. In particular, the equations governing the SHH ligand and PTCH1 receptors are identical. Thus, there are not enough equations to uniquely determine a steady state, if one does exist. These difficulties can be overcome by investigating the dynamics of the system via numerical integrations in time. To begin such a simulation, it is assumed that there is an initial abundance of free SHH ligands and PTCH1 receptors. Furthermore, we assumed that all of the SMO was in the inactive state. Mathematically, these initial conditions correspond to

$$x_1(0) = 2, \quad x_2(0) = 1, \quad x_3(0) = 0, \quad x_4(0) = 1, \quad \tilde{x}_4(0) = 0.$$

As a preliminary investigation into the dynamics of the system, we first neglect the effects of drugs. The results from such a time integration can be seen in Figure 4. This figure shows the evolution of the various receptors and ligands, and it also displays the proliferation of cancer cells. From this figure, it can be seen that the SHH ligands quickly bind to the free PTCH1 receptors and reach a quasi-steady state almost immediately. Due to the positive feedback loop described above (see Section 2), the steady state is not actually reached because as long as there are some active SMO ligands, a series of biological processes will occur which eventually leads to generation of more SHH and PTCH1 ligands. The amount of cancerous cells, which is quantified using (3), appears to exhibit exponentially fast growth. This results from the fact that the amount of active SMO remains essentially constant in time, which from a biological standpoint, implies a constant inhibition of the P53 tumour suppressor gene. Furthermore, this figure depicts an increasing amount of SHH/PTCH1 complexes. These excess complexes can be thought of as inactive, since they do not appear to be activating additional SMO ligands (which do lead to cell proliferation). It then follows that if a drug treatment were to target these complexes after they have formed, the treatment would be highly ineffective at reducing cell proliferation because the drugs would have the effect of reducing the “inactive” complexes. Instead, the SMO ligands should be targeted directly, if possible.

The effects of targeting different parts of the pathway can be studied theoretically by performing various time integrations with different drug concentrations and recording the amount of cancer at the final time. As mentioned previously, drugs are introduced into the equations by assuming they only vary the rate constants of the interactions. We chose to focus on drug treatments which affect either the formation of the SHH/PTCH1 complex, or the positive feedback loop created by the active SMO ligands. The results can be seen in Figure 5. This figure shows the amount of cancer at the final time (taken to be 10 units of time) as a function of the amount of drug, where the drug amount has been normalized so that an amount of unity corresponds to completely stopping the given process. As predicted, we find that targeting the SMO feedback loop is far more effective than inhibiting the formation of the SHH/PTCH1 complex. However, Figure 5 must be interpreted with caution. For instance, it is not clear how much drug is actually needed to completely inhibit these two processes, and these amounts could differ by orders of magnitude. However, the important

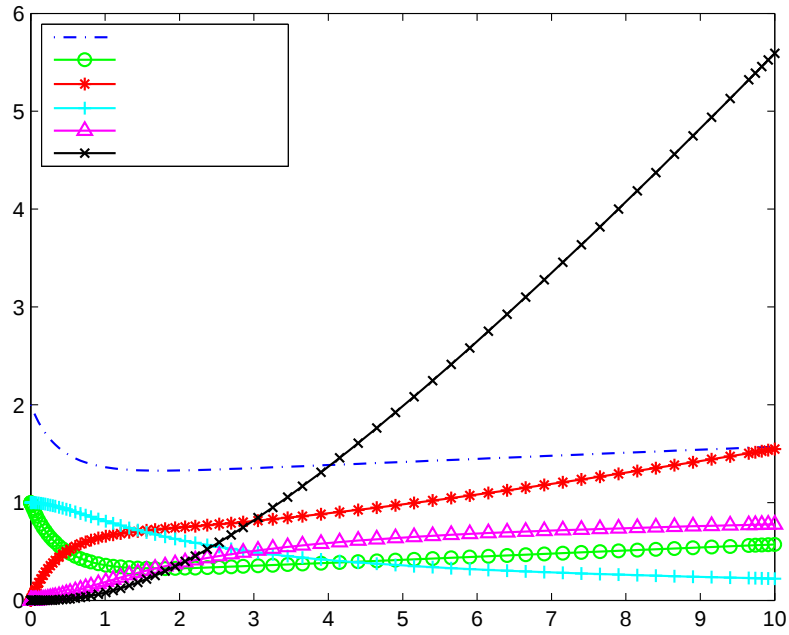


Figure 4: Evolution of SHH ligands (line/dots), PTCH1 receptors (line/circles), SHH/PTCH1 complexes (line/star), inactive and active SMO receptors (line/plus and line/triangle, respectively). Also shown is the growth of cancer cells. The effects of drugs are not considered here.

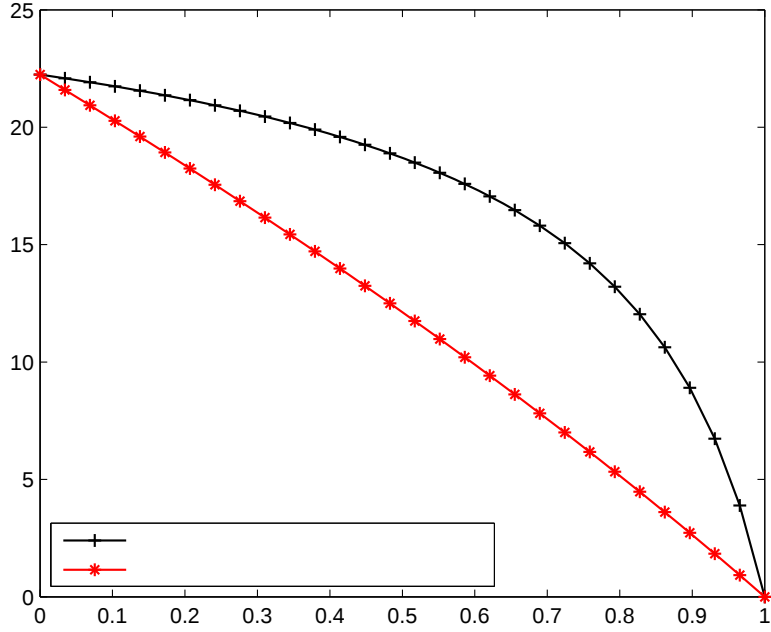


Figure 5: The effect of a chemotherapeutic treatment can be determined by measuring the amount of cancer at some time as a function of the drug concentration. We considered two treatments; one of which focused on inhibiting the formation of SHH/PTCH1 complexes (line/plus) and the other which inhibits the SMO feedback loop (line/star).

point is that targeting different interactions in the pathway can significantly improve the efficacy of a treatment.

4 Discussion and Conclusions

In this report we developed a small set of ordinary differential equations that model a simplified version of the basal cell carcinoma pathway. This model looked at how the constituent ligands and receptors in the pathway interact with one another and it incorporated the effects that drug treatments would have on these various interactions. By performing numerical experiments on the differential equations, it was found that targeting different interactions can have a significant effect on the amount of cell proliferation that occurs in a given time. In particular, our model suggests that targeting the positive feedback loop between activated SMO, PTCH1, and SHH, is much more effective at reducing the number of cancerous cells than using a treatment which inhibits the formation of SHH/PTCH1 complexes. Unfortunately, our model uses unrealistic parameter values and therefore it cannot be used in its current form to make predictions about real life treatments. However, it does show if one can accurately model the key interactions in a biological pathway, then one can use the model to investigate the interactions in the pathway that are essential for cell proliferation. Using this information, a treatment can be created which focuses on targeting this critical interaction. Furthermore, this report shows the type of approach that can be taken in order

to build such models. In fact, it could be possible to create a generic toolbox of equations that can be used to string together various types of interactions, such as a ligand binding to a receptor followed by an activation via phosphorylation. When armed with such a toolbox, one could build arbitrarily complex models of biological pathways.

Of course, modelling the role of drugs in a biological pathway is not as simple as we have described, as we have neglected the very important fact that the drugs which are used in cancer treatments are usually damaging to healthy cells as well. Therefore, an ideal model would have to take this fact into account. One possible way of doing this would be to study an optimization problem that aims to minimize both the amount of cancerous cells at some terminal time and the total amount of drug that is used during the treatment. There are countless other methods in which this problem can be modelled, but one fact remains the same: the analysis of accurate mathematical models can, in theory, be used to develop new treatments that are both highly effective and allow the patient to live with a high quality of life.

References

- [1] World Health Organization. Annex Table 2 Deaths by cause, sex and mortality stratum in WHO regions, estimates for 2002. *The world health report 2004*. 2004. Available at http://www.who.int/entity/whr/2004/annex/topic/en/annex_2_en.pdf
- [2] M. Athar, X. Tang, J. L. Lee, L. Kopelovich, A.L. Kim, Hedgehog signalling in skin development and cancer, *Exp. Dermatol.* **15** (2006), 667-677.
- [3] L. Daya-Grosjean, S. Couvé-Privat, Sonic hedgehog signaling in basal cell carcinomas, *Cancer Letters* **225** (2005), 181-192.
- [4] G. Saldanha, The Hedgehog signalling pathway and cancer, *J. Pathology* **193** (2001), 427-432.
- [5] C.M.L.J. Tilli, M.A.M. Van Steensel, G.A.M. Krekels, H.A.M. Neumann and F.C.S. Ramaekers, Molecular aetiology and pathogenesis of basal cell carcinoma, *J. Dermatol.* **152** (2005), 1108-24.
- [6] <http://www.genome.jp/kegg/pathway.html>