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IDENTIFYING POTENTIAL GENE THERAPY TO TREAT GLIOBLASTOMA THROUGH INHIBITION OF THE PI3K/AKT/MTOR SIGNALING PATHWAY USING FUZZY STOCHASTIC HYBRID FUNCTIONAL PETRI NETS

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Abstract. Glioblastoma multiforme is a common and a cancerous primary brain tumor. Despite of the existence of many known treatments, such as chemotherapy, radiation therapy, and even surgical resection, the survival rate of Glioblastoma multiforme patients is significantly low and alarming. Therefore, discovering new treatments of the disease is essential. One of the signaling pathways under consideration in glioblastoma can be PI3K/AKT/mTOR, which can be up-regulated through various mechanisms. In the present study, Fuzzy Stochastic Hybrid Functional Petri net was used as a mathematical tool to model PI3K/AKT/mTOR signaling pathway. The simulation results showed that protein kinase B (Akt) short hairpin RNA gene therapy decreases the level of glioblastoma multiforme significantly and can be used as a potential gene therapy to treat brain cancer.

Keywords: Glioblastoma, gene therapy, Petri nets, quantitative modelling.
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1 Introduction

Glioblastoma multiforme (GBM) is a common primary brain tumor, which have aggressive malignant property because of their fast cell growth and their linkage to blood vessel network. These brain tumors can be located in different parts of brain or even in the spinal cord. However, the most common location of GBM is found to be in the cerebral hemispheres of the brain (Fernando et al., 2018). The pathogenesis of GBM is very complex, which includes mutations and alterations of several key cellular pathways (Pearson, 2017). The current known pathways, developing GBM are PTK/Ras/PI3K (Pearson, 2017), RAS/MAP/ERK (Kolch et al., 2000), and PI3K/Akt/mTOR (Polivka et al. (2008)). PI3K/Akt/mTOR is activated by several receptors, including G-protein-coupled, tyrosine kinase growth factor, and transmembrane integrins (Pearson, 2017). The activation of PI3K/Akt/mTOR pathway leads to phosphatidylinositol 3,4,5-triphosphate (PIP3) production, which activates Protein kinase B (Akt), and eventually phosphorylation of Akt and Phosphoinositide 3-kinase (PI3K) becomes one of the main factors in the formation of Glioblastoma (Polivka et al., 2008). In addition, Akt directly and indirectly activates mTOR which helps in the formation and growth of Glioblastoma by activation of Ribosomal protein S-6 (which upregulates p70s6k and cooperates with RAS/MAP/ERK pathway) and SGRP (Kolch et al., 2000; Pearson, 2017; Polivka et al., 2008).

There exist several treatments for GBM, such as chemotherapy, radiation therapy, proton therapy, and surgery. Choosing the proper strategy to treat GBM is crucial since it depends on many factors such as size, type, and location of the tumor. In addition, the response of the

treatment differs from a patient to another patient based on their health condition, age, and unknown personalized factors. Although there have been significant improvement of GBM treatments, this disease is still among difficult cancers to treat. Therefore, identifying new strategies to optimally manage GBM disease is urgent. PI3K/AKT/mTOR pathway can be upregulated through various mechanisms, and inhibiting or altering this signaling pathway may lead to the development of potential molecular targets to find novel GBM treatments (Polivka et al., 2008). Protein kinase B (PKB), which is also known as Akt can be activated by phosphatidylinositol 3,4,5-triphosphate (PIP3). PIP3 itself, can be generated by PI3K enzyme. Thus, PI3K plays an important role to activate Akt. Downstream of mammalian target of rapamycin (mTOR) can receive signals from Akt, which can also activate P70 ribosomal S6 (p70S6K). Therefore, PI3K/Akt/mTOR caught the attention of scientists as a significant signaling pathway in tumor cells, such as in GBS (Li et al., 2016). Therefore, other therapies such as short hairpin RNA gene therapy should be evaluated as an alternative for knocking down Akt gene expression (shAkt). On the other hand, the role of phosphatase and tensin homologue gene (PTEN) seems to be important as the lack of its expression can result in activation of PI3K/Akt/mTOR signaling pathway (Pitter et al., 2011). Therefore, creating a model to describe PI3K/Akt/mTOR signaling pathway is essential.

A Petri net is a directed graph including of places, transitions, and arcs, which connects places/transitions to transitions/places. The number of parallel arcs between any places or transitions can be indicated by arc weights. A place in a Petri net represents entities of the system, and the flow of tokens among these places shows the dynamic structure of a Petri net. The distribution of the tokens in places of a Petri net is called marking of a Petri net. In the last few decades, many new properties have been added to the classical Petri nets to describe distributed systems more accurately. For example, Continuous Petri nets were introduced to include continuous places and transitions in a Petri net. However, most of the biological systems include discrete and continuous entities. Therefore, hybrid Petri nets were introduced to include discrete and continuous places (David & Alla, 2001). Activation of a certain transition in a Petri net depends on firing rules. Whenever the condition of firing rules in a transition holds, that transition can "fire". That is, the transition removes the tokens from its input places, and adds them to its output places. Hybrid Petri nets with such firing rules on their transitions are called Hybrid Functional Petri nets (Matsuno et al., 2003). Simulation results obtained by a single run in deterministic Hybrid Functional Petri nets can be used as proper estimations to describe complex systems such as biological systems (Mehraei et al., 2016). However, it is not possible to measure the accuracy of the simulation results of such quantitative modeling. Therefore, some extended properties have been added to Hybrid Functional Petri nets, such as color, time, stochastic, and fuzzy extensions (Liu et al., 2016).

In this study, PI3K/Akt/mTOR is mathematically modeled using Fuzzy Stochastic Hybrid Functional Petri nets (FSHFPNs). This model made it possible to analyze PI3K/Akt/mTOR signaling pathway, and to identify novel gene therapy for GBM.

2 Method

2.1 RNAi-mediated gene knock down

RNA interference (RNAi) is a biological phenomenon which knocks down the gene expression using its own DNA sequence. The current approaches of RNAi-medated gene knock down are small interfering RNA (siRNA), short hairpin RNA (shRNA), and micro RNA (miRNA). The first step in RNAi approach is to cut of long double stranded RNA (dsRNA) into short fragments of siRNAs (Usually between 20-25 number of fragments). These fragments of siRNAs enter the cell and is incorporated into RNA-induced silencing complex, which separates its strands. In the next step, a guided strand pairs with a complementary sequence in mRNA let the subsequent cleavage of the target mRNA. This action leads to turning of target gene

expression, and accumulation of protein chains. Removing the unnecessary fragments creates siRNAs in the cytoplasm by the exportation of expressed shRNA in the nucleus into cytoplasm.

2.2 Quantitative modeling with FSHFPN

Petri nets modeling is a useful approach to describe biological systems (Mehraei et al., 2016). It provides this chance to make predictions by manipulating the transition rates without no cost unlike wet lab experiments. In the current study, the proposed FSHFPN model (Stochastic Hybrid Functional Petri nets with Fuzzy parameters) was conducted in terms of quantitative modeling with stochastic Petri nets on the Snoopy platform (Heiner et al., 2000). This model contains both discrete and continuous places. Therefore, Hybrid Petri nets were needed to create the model (David & Alla, 2001). In the proposed model, continuous places represent DNAs, mRNAs, proteins, and multi-proteins. In addition, discrete places were used for indicating the presence/absence of PTEN mutation and Perifosine drug. There exist firing rules, and inhibitory arcs in this model, so the model need to use hybrid functional Petri nets. Continuous transitions were used to decrease the level of PTEN and phosphrylated Akt (pAkt). Perifosine inhibits Akt phosphrylation and its activation (Pitter et al., 2011). The transition rates of these continuous places were chosen the way to decrease the level of pAkt by approximately 24% and 43% in PTEN-intact and PTEN-deficient, respectively (Pitter et al., 2011). A segment of the proposed Petri net model related to presence/absence of Perifosine drug is illustrated in Figure 1. The discrete place of presence/absence of Perifosine can take either 1 or 0 values. When the marking of this place is 0, it means the cells are under Perifosine treatments. Otherwise, it means the cells are left untreated.

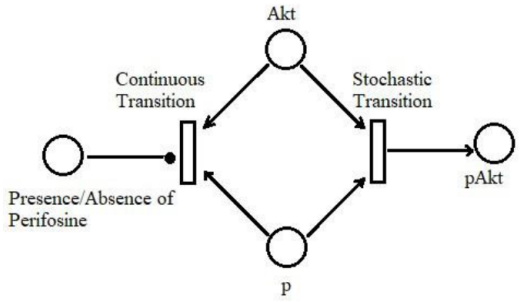

Figure 1: Inhibition of pAkt through Perifosine in FSHFPNs model

Generally, biological systems are governed by stochastic aspects. Therefore, to model biological systems, stochastic property should be added to hybrid functional Petri nets (Heiner et al., 2008). In the proposed model, the processes related to activation, transcription, translation, natural degradation, and binding were considered using stochastic transitions. All mRNAs and proteins in this model are following the central dogma of biology, and their levels are kept steady using natural degradation. The central dogma of biology and natural degradations in the Petri net model are illustrated in Figure 2.

It is almost impossible to decide about exact kinetic parameters in a mathematical model since wet lab observations tend to lead to different or sometimes contradictory results. Therefore, determining values as kinetic parameters is the most challenging part of creating quantitative models such as Petri nets. In complex biological systems, most of kinetic parameters are either uncertain or unknown. Therefore, Fuzzy Stochastic Petri nets (FSPNs) were introduced to overcome this problem using fuzzy sets with stochastic Petri nets (Liu et al., 2016). In this study, unknown or vague kinetic parameters were considered as fuzzy numbers. The summary of transition rates is illustrated in Table 1. The transition rates were assigned using similar biological pathways (Mehraei & Bashirov, 2019), reverse engineering technique (Mehraei et al., 2016), and arbitrary fuzzy parameters (Mehraei, 2018).

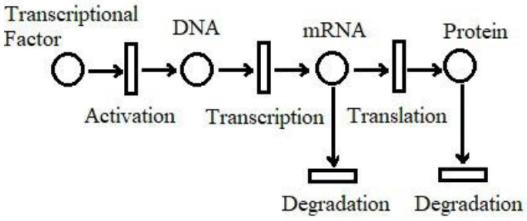

Figure 2: Central dogma and natural degradation in the proposed Petri nets model

Table 1: Transition rates in the proposed FSHFPN model.

Process	Type of transition	Rate of transition
Transcription	Stochastic	K1=(0.09, 0.1, 0.11)
Translation	Stochastic	K1
Activation	Stochastic	K1
Binding	Stochastic	K1
mRNA degradation	Stochastic	K1
Protein degradation	Stochastic	K2=(0.009, 0.01, 0.011)
Perifosine process	Continuous	K3=(0.05, 0.06, 0.075)
PTEN-mutation process	Continuous	K4=(6.5, 7, 7.8)
shAkt process	Continuous	K5=(999.2, 1000, 1001)

The main structure of the proposed FSHFPN model is illustrated in Figure 3. To simplify the illustration, this figure does not contain the parts related to central dogma of biology and natural degradations which were already shown in Figure 2.

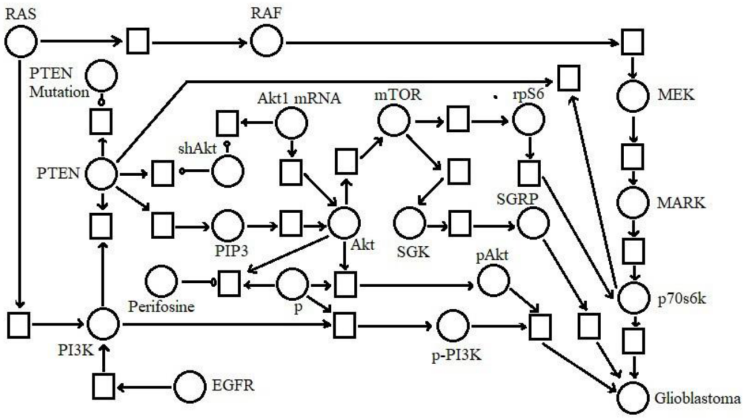

Figure 3: Main structure of the proposed FSHFPNs model

3 Results

The simulation results were obtained by finding the average mean of 40000 stochastic simulation runs on Snoopy software tool (Heiner et al., 2012) with p-value less than 5% based on the formula proposed by Sandmann and Maier (Sandmann et al., 2008). The fuzzy numbers for each places were considered as triangular format. Therefore, to obtain the simulation results in terms of fuzzy numbers, the simulation results were calculated by considering three α cuts. One can neglect the accuracy by decreasing the number of simulation runs (Liu et al., 2016), but the

40000 number of runs made it possible to have the coefficient of variation tending to 1 in Sandman and Maire's formula (Sandmann et al., 2008). The results are measured at 500 Petri time (pt) when the concentration levels of each place are at steady stage. All the simulation results were measured at 500 pt to make the comparison between treated and untreated cases.

The average mean of 40000 stochastic simulation runs at 500 pt showed that shAkt gene therapy decreased Akt1 mRNA level by approximately 99%. In addition, pAkt level was decreased by approximately 1.3-fold, 1.8-fold, and 6.6-fold in Perifosine drug-based treatment, Perifosine treatment along with mutated PTEN, and shAkt gene therapy comparing with untreated control, respectively. FSHFPN simulation results of pAkt are illustrated in Figure 4.

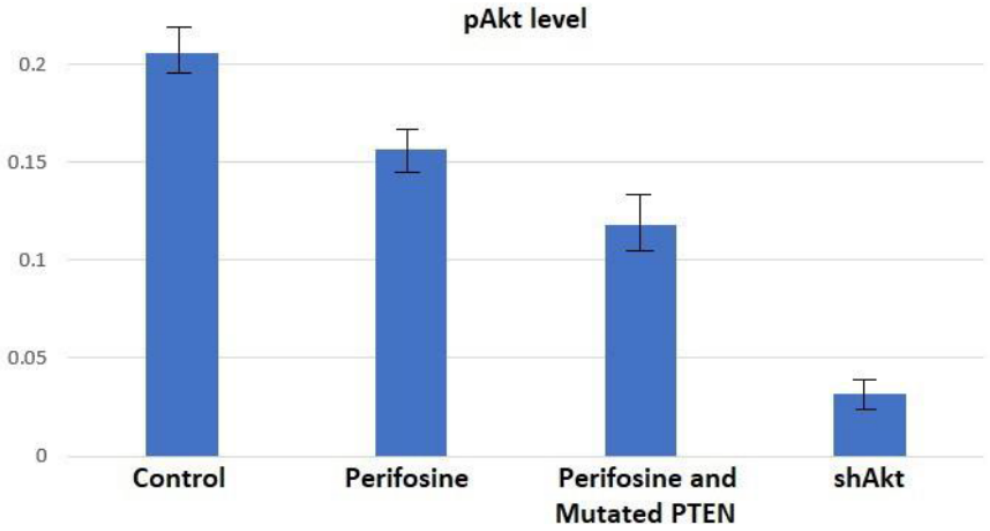

Figure 4: Simulation results of pAkt level at 500 pt.

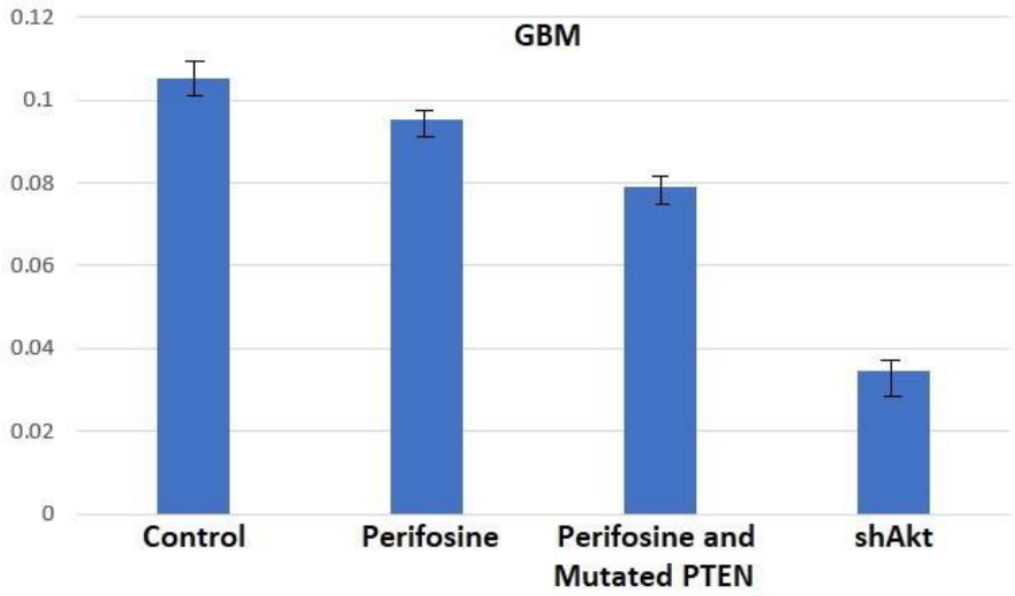

Figure 5: Simulation results of GBM level at 500 pt.

GBM level was measured by calculating the average mean of 40000 numbers of stochastic simulation runs at 500 pt for untreated control case and different treatment strategies. The simulation results revealed that the level of GBM was decreased by approximately 1.1-fold, 1.3-fold, and 3-fold in Perifosine drug-based treatment, Perifosine treatment along with mutated

PTEN, and shAkt gene therapy compared with untreated control, respectively. Therefore, shAkt can significantly decrease the level of GBM with 95% confidence using hypothesis testing based on Sandmann and Maier's formula (Sandmann & Maire, 2008) with 0.05 as its p-value, and can be used as a potential gene therapy to treat brain cancer. FSHFPN simulation results of GBM are illustrated in Figure 5.

4 Conclusion

The present research uses FSHFPN as a quantitative modellng approach to identify a potential gene therapy scenario to treat Glioblastoma through inhibition of the PI3K/AKT/mTOR signaling pathway. In the meanwhile, it is shown that how quantitative modeling with FSHFPN can be used to shed light on how complex biological systems can be described and analyzed. The average mean of 40000 stochastic simulation runs with 95% confidence level showed that silencing Akt1 gene using shAkt RNAi-mediated approach can effectively decrease the level of GBM much more comparing to known drug-based treatments through inhibition of the PI3K/AKT/mTOR signaling pathway. Therefore, shAkt can be used as a novel gene therapy to effectively treat brain cancer. Although it is out of the scope of this paper, as future studies in vivo, further discussion of biological consequences at both molecular level and the phenotype should be considered in collaboration with molecular biologists and Pharmacogenetics groups.

In future studies, the current proposed FSHFPN model can be extended to describe other signaling pathways in GBM to find potential molecular targets to identify both novel drug-based and gene therapy strategies to treat brain cancer, or at least decrease the size of brain tumors significantly.

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