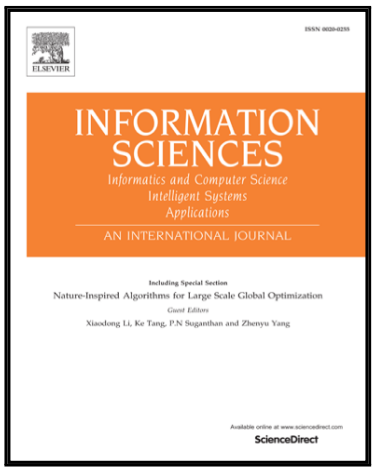

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Identifying targets for gene therapy of β-globin disorders using quantitative modeling approach

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Abstract

Sickle cell disease and β-thalassemia are well-known genetic disorders which are caused by mutations in β-globin gene. Reactivation of fetal hemoglobin in adulthood through induction of γ-globin gene expression has proven be sufficient to ameliorate sickle cell disease and β-thalassemia. In the last few decades, substantial efforts have been made to identify potential target candidates for β-globin diseases. In the present work, we propose an innovative approach for identifying novel molecular targets of β-globin diseases. Our approach is based on quantitative modeling of fetal-to-adult hemoglobin switching network with hybrid functional Petri nets. We verify the coherence of deterministic quantitative model created in this research to be sure it is consistent with qPCR data available for known siRNA- and shRNA-based strategies. Comparison of simulation results for the proposed strategy with the ones obtained for already existing RNAi-mediated treatments shows that our strategy is optimal, as it leads to the highest level of γ-globin induction. Consequently, it has potential beneficial therapeutic effect on β-globin diseases. This study also provides an innovative strategy for the target-based treatment of many other diseases.

Keywords: Hybrid functional Petri net; Quantitative modeling; Cell Illustrator; β-thalassemia; Sickle cell disease; Fetal-to-adult hemoglobin switching network

1. Introduction

Sickle-cell disease (SCD) and β-thalassemia are among major sources of mortality and morbidity in the world [27]. The major curative therapies which currently exist for SCD and β-thalassemia include bone marrow transplantation [20], gene therapy [21], and transfusion of red blood cells [22]. Numerous challenges encountered in the practical implementation of curative therapies are discussed in [7,22]. These challenges essentially complicate widespread use of curative therapies; therefore, researchers have eventually moved beyond the challenges, searching for alternatives to curative therapies.

Fetal hemoglobin (HbF) remains as the major hemoglobin during fetal life, but it diminishes to 1% of total hemoglobin soon after birth, gradually being replaced by adult hemoglobin (HbA) during infancy. The defective or insufficient production of β-globin chains in HbA due to diverse point mutations, that is a major cause of SCD and β-thalassemia, can be essentially replaced by an increase of γ-globin chains in HbF. The recent advances in hemoglobin biology suggest that even modest induction of HbF in infancy may be sufficient to compensate the

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deficit in HbA and consequently alleviate the severity of SCD and β-thalassemia [28,29].

Over the past few decades there has been growing interest in developing target-based approaches for induction of γ-globin gene and HbF expression. Novel target-based approaches for treatment of β-globin diseases include drug therapy and RNAi-based gene therapy. Although both approaches begin with the identification of a specific molecular target involved in silencing of γ-globin gene, in the former approach, a drug that is effective on the selected target is then used to silence the target, while the latter one uses RNAi-mediated mechanism to knockdown the target with high specificity and selectivity. Although they use different techniques to inhibit the targets, both approaches result in the induction of γ-globin gene levels. The most critical concern regarding drug therapy remains as on-target and off-target side effects. RNAi-based techniques on the other hand have no adverse side effects that are encountered for drug therapy, as they ensure the selective destruction of only a target, so that in absence of suitable target RNAi-mediated gene therapy remains inert causing no effect within the cell. This is the main advantage of RNAi-mediated gene therapy over drug therapy.

Improved understanding of molecular interactions in fetal-to-adult hemoglobin switching mechanism holds the key to the identification of novel therapeutic targets not only for SCD and β-thalassemia but also for other β-globin disorders. The importance of B-cell lymphoma/leukemia 11A (BCL11A), Kruppel-like transcription factor 1 (KLF-1), myeloblastosis (Myb), SOX6 and histone deacetylase 1 and 2 (HDAC1/2) as the drug targets for induction of γ-globin gene in humans is discussed in [25]. A number of target-based drugs currently available or in clinical trials have been tested for induction of γ-globin gene [9,11,17,23,26]. In [19], using Petri net involved quantitative modeling we compare the effect of these drugs on the induction of γ-globin gene and show that multiprotein complex of Erythroid Transcription Factors (ETF) turns out to be more efficient drug target as its silencing leads to greater induction of γ-globin gene compared to the ones discussed in [9,11,17,23,26].

In this paper, we explore the comparative efficacy of five RNAi-mediated gene therapeutic strategies for inducing γ-globin gene expression: (1) reducing methyl-binding domain (MBD2) mRNA expression by siRNA-mediated knockdown of MBD2 [13], (2) shRNA-mediated knockdown of Myb followed by silencing of KLF-1 and BCL11A mRNAs [24], (3) shRNA-mediated knockdown of BCL11A followed by silencing of KLF-1 and BCL11A mRNAs [24], (4) siRNA-mediated knockdown of chromodomain helicase DNA binding protein (CHD4) followed by silencing of KLF-1 and BCL11A mRNAs [3], and (5) our proposed RNAi-mediated strategy of inhibiting BCL11A, friend of GATA protein 1 (FOG1) and HDAC1/2 mRNAs. Simulation results show that our strategy is the optimal one among five strategies discussed in the present work as it identifies the rational molecular targets yielding the greatest induction of γ-globin gene levels. Moreover, comparison of simulation results obtained with our strategy with the drug therapeutic approaches presented in [19] shows that our strategy also improves results discussed in [19] on induction of γ-globin levels.

The present research is conducted in terms of quantitative modeling using Petri net technologies. We use Cell Illustrator software to create the closest model of molecular interactions in fetal-to-adult hemoglobin switching network, then use available qPCR data to validate the model for siRNA and shRNA effects in accordance with strategies (1)-(5), and finally identify the optimal molecular targets for RNAi-mediated gene therapy of β-globin disorders.

2. Materials and Methods

2.1. RNAi-mediated gene knockdown approach

The discovery of RNAi in 1998 [12] became a substantial event in molecular biology in the past two decades. RNAi is a post-transcriptional reverse genetic process that uses the gene's own DNA sequence to turn it off. The advent of RNAi made it possible to turn off almost every gene in human cells. The key approaches of mediating the RNAi effect involve small interfering RNA (siRNA) and short hairpin RNA (shRNA).

During RNAi, long double stranded RNA is cut into siRNA molecules each of which is typically 20-25 nucleotide in length. Then selected siRNA enters into the cell and binds to RNA-induced silencing complex, which in turns separates its strands. Following this, a separated strand binds to the target mRNA with complementary sequence, resulting in subsequent degradation of a target mRNA or suppression of its translation. This, in either case, stops further protein production, leading to a decrease in its levels, and eventual knockdown. While both

siRNA and shRNA can be used for gene silencing, there are differences in their mechanisms of action. After expression in the nucleus, shRNA is exported to the cytoplasm where it is converted into siRNA by removal of unnecessary fragments. From this point, they are processed in the same manner as siRNAs.

2.2. Fetal-to-adult hemoglobin switching mechanism

Understanding of the human fetal-to-adult hemoglobin switching mechanism holds the key to the identification of new molecular targets for gene therapy of SCD and β-thalassemia. It was observed that shRNA-mediated knockdown of BCL11A and Myb separately result in derepression of γ-globin gene [24]. NuRD, nucleosome remodeling deacetylase complex of HDAC1/2, MDB2 and CHD4 (Mi2β), and HDAC1/2, contributes to γ-globin gene silencing by binding to BCL11A. It has been shown that siRNA-mediated knockdown of MBD2 results in the increase of γ-globin gene expression [13]. CHD4 is another critical component of NuRD multiprotein complex. CHD4 binds directly to and positively regulates both the KLF1 and BCL11A genes that are critical regulators of γ-globin gene. It has also been observed that siRNA-mediated knockdown of CHD4 significantly induces γ-globin gene expression [3]. SOX6, GATA1 and FOG1 are Erythroid Transcription Factors that contribute to repression of γ-globin gene expression by binding to BCL11A. Molecular interactions between regulators of fetal-to-adult hemoglobin switching mechanism are illustrated in Fig.1. Additionally, HDAC1/2 as the component of NuRD cooperates with BCL11A to silence γ-globin gene.

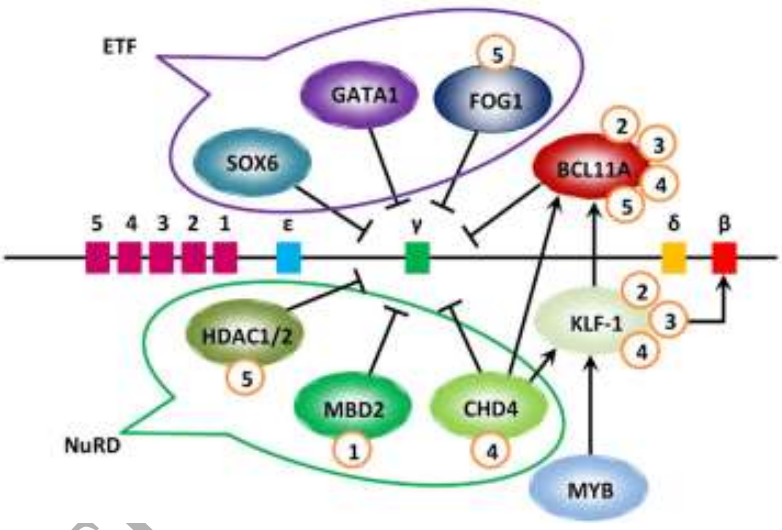


Fig. 1: The positive and negative molecular interactions between Myb, KLF1, BCL11A, CHD4, MBD2, HDAC1/2, SOX6, GATA1, FOG1, the main molecular regulators of fetal-to-adult hemoglobin switching, and γ- and β-globin genes are respectively represented by regular and blunted arrows. A number inside a circle surrounding biological component stands for the RNAi-mediated strategy that affects specified biological component.

2.3. Quantitative modeling with Petri nets

Biological systems are characterized by rapid molecular interactions between genes, mRNAs, proteins and their complexes. It is quite regular to express, interpret and predict the characteristics of biological systems in terms of quantitative change in concentration level of biological components. Constructing quantitative models of biological systems is therefore crucial to make meaningful deductions regarding the behavior of biological systems.

Over the last two decades Petri net technologies have been extensively used for creating quantitative models of metabolic networks, signal transduction pathways and gene regulatory networks. When modeling biological processes, Petri net components such as entity (or place), process (or transition) and arc are respectively used to represent corresponding biological component, biological phenomena and flow of biological information. Concentration, reaction rate and reaction stoichiometry are assigned as parameters to places, transitions and arcs. Biological systems usually comprise continuous, Boolean and discrete processes. Corresponding Petri nets are

expected to be continuous [10], hybrid [2] and functional [18]. Numerous problems arising in engineering and scientific domains have been successfully modeled and simulated in terms of Petri net technologies [4,5,8,14-16]. Particularly, we have used hybrid functional Petri net (HFPN) to create a quantitative model of molecular interactions between major regulators of fetal-to-adult hemoglobin switching network [19] and p16-mediated signaling pathway [1].

3. Results

3.1. Construction of the model

In this paper, we create HFPN model of fetal-to-adult hemoglobin switching network based on qPCR data retrieved from [3,13,24]. Screenshot of Cell Illustrator demonstrating HFPN model is represented in Fig.2 while skeleton of the model is depicted in Fig.3. In Fig.3, for the sake of clarity we focus on binding, mRNA activation and suppression, discarding protein production by central dogma of biology and degradation though HFPN model in Fig.2 incorporates all these processes.

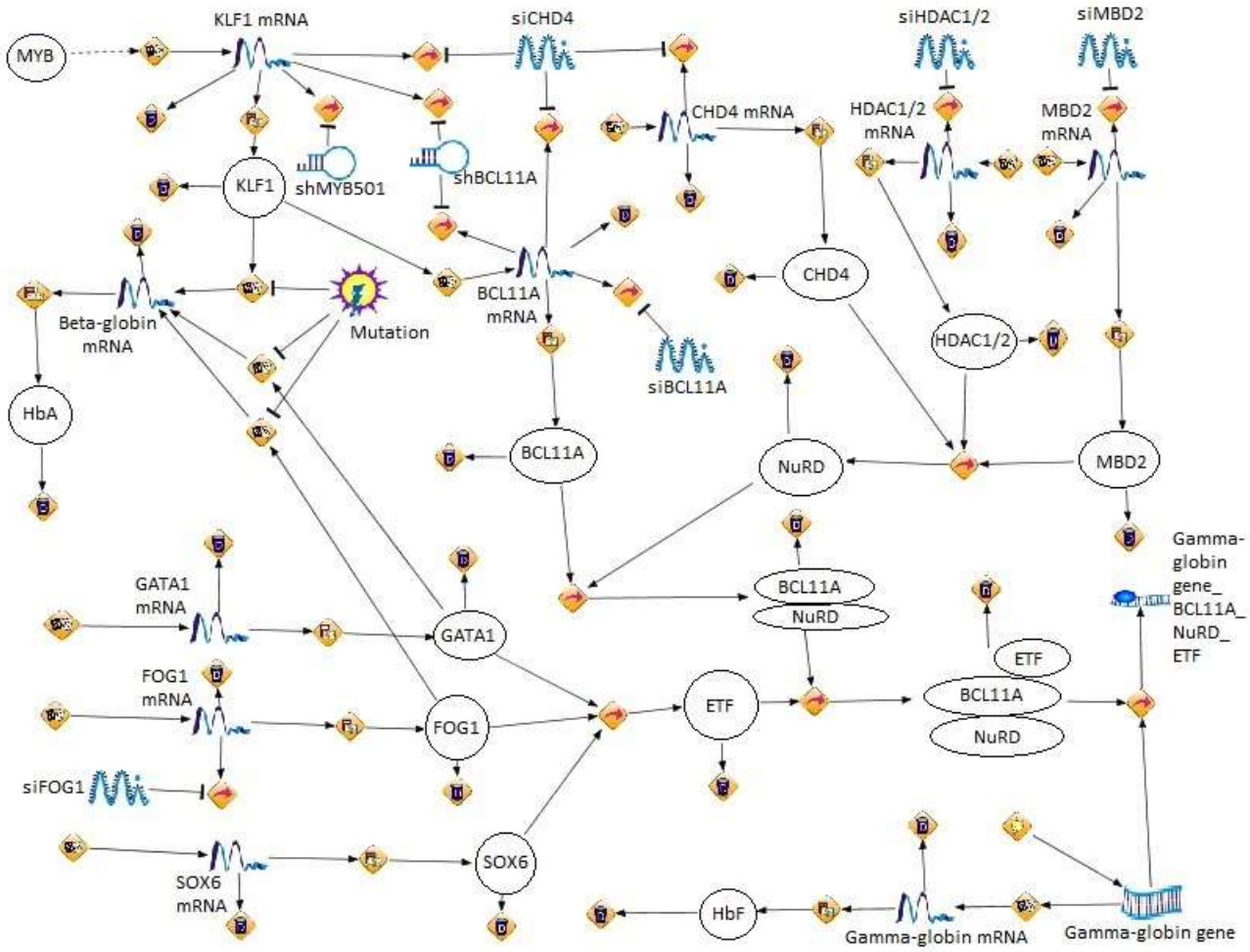


Fig. 2: Screenshot of HFPN model of fetal-to-adult hemoglobin switching network.

Our HFPN model is composed of 8 generic entities, 27 continuous entities, 61 processes, and 114 arcs. The generic entities represent siRNA and shRNA components of Myb, BCL11A, MBD2, CHD4, HDAC1/2, FOG1 and β-globin gene mutation. Continuous entities stand for γ-globin gene, mRNAs, proteins, and their complexes. The processes

include transcription, translation, binding and degradation. Boolean variables are used to check true/false condition for siRNA and shRNA components and β-globin gene mutation. The model includes inhibitory arcs in addition to regular ones. Details of the model can be found in Tables 1-4.

In our model, we assume that a protein is produced in accordance with the central dogma of biology. This is why, the initial concentration for all continuous places are set to 0 (see data under "value" in Table 1). Since the proteins are produced in accordance with the central dogma of biology, transcription of DNA into mRNA is represented by source transition, which continuously feeds mRNA and consequently protein. Initial concentration of a discrete place is either 0 or 1, indicating the presence or absence of corresponding RNAi-based treatment. In order to indicate the presence of a treatment, we used 0 for such Boolean places, where there is an inhibitory arc to the process, which leads to further degradation of a targeted component.

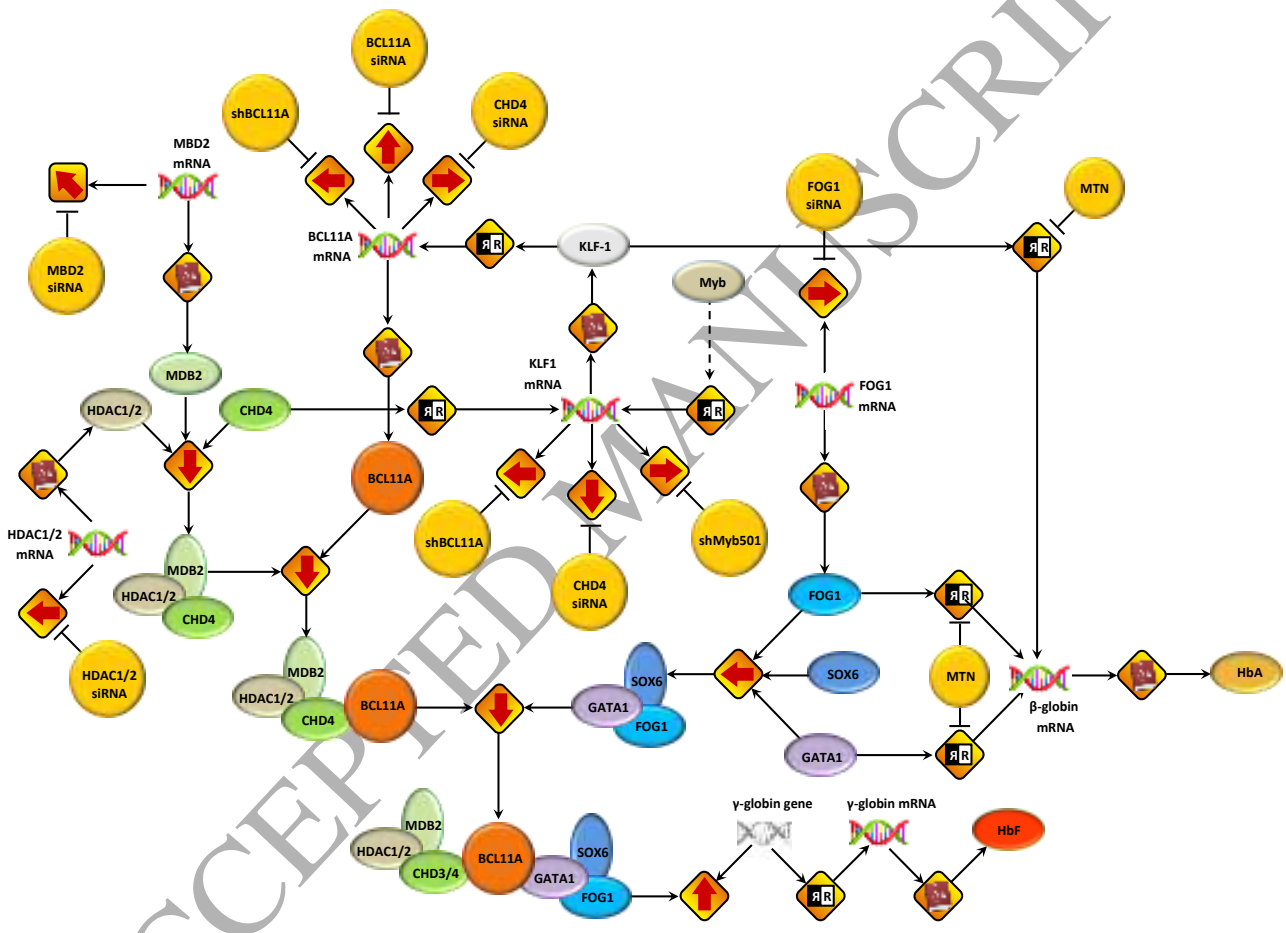


Fig.3: HFPN model of fetal-to-adult hemoglobin switching network.

It is quite often the case that due to external conditions two identical wet lab experiments lead to not identical observations. Because of this reason, it is hard, if not impossible to determine the reaction rates solely based on wet lab observations. Transcription and translation rates are adopted from [1,18,19]. Those which are not following the routine process rates are obtained by applying reverse engineering method in order to reach the closest fit for hemoglobin switching developmental process. In this research we use wet lab observations for siRNA- and shRNA-based treatments as a reference to compare in silico results to be validated with those obtained in wet lab experiments. Then we carefully calibrate in silico results wherever and whenever possible to have clear trend and similarity between in silico results and qPCR data in [3,13,24]. The process rates adopted in this work are indicated in Tables 2-3.

3.2. Numerical validation of the model

In this research we use Cell Illustrator 5.0 as computational platform to construct HFPN model of human fetal-to-adult hemoglobin switching mechanism, validate the model, perform comparative analysis of strategies (1)-(5) described in Section 1 in order to determine an optimal RNAi gene therapeutic strategy derepressing γ-globin production.

We plot the concentrations (on the y axis) versus time (on the x axis) measured in Petri time or pt, for short, with the time interval of 10 pt corresponding to 3 months of gestational age. In these plots, it is assumed that fetal life starts at 20 pt (0 months) and child is born at 50 pt (9 months). We carry out measurement of a concentration at 70 pt, when its level reaches stable steady state.

Table 1. Entities in the HFPN model of human fetal-to-adult hemoglobin switching network.

Entity name	Entity type	Variable	Initial value	Variable Type
Myb	Continuous	m1	1	Double
KLF-1mRNA	Continuous	m2	0	Double
KLF-1	Continuous	m3	0	Double
BCL11AmRNA	Continuous	m4	0	Double
BCL11A	Continuous	m5	0	Double
HDAC1/2mRNA	Continuous	m6	0	Double
HDAC1/2	Continuous	m7	0	Double
MDB2mRNA	Continuous	m8	0	Double
MDB2	Continuous	m9	0	Double
CHD4mRNA	Continuous	m10	0	Double
CHD4	Continuous	m11	0	Double
NuRD	Continuous	m12	0	Double
BCL11A_NuRD	Continuous	m13	0	Double
GATA1mRNA	Continuous	m14	0	Double
GATA1	Continuous	m15	0	Double
FOG1mRNA	Continuous	m16	0	Double
FOG1	Continuous	m17	0	Double
SOX6mRNA	Continuous	m18	0	Double
SOX6	Continuous	m19	0	Double
ETF	Continuous	m20	0	Double
BCL11A_NuRD_ETF	Continuous	m21	0	Double
γ-globin_BCL11A_NuRD_ETF	Continuous	m22	0	Double
γ-globin gene	Continuous	m23	0	Double
γ-globin_mRNA	Continuous	m24	0	Double
HbF	Continuous	m25	0	Double
Mutation	Generic	m26	0	Boolean

β-globin_mRNA	Continuous	m27	0	Double
HbA	Continuous	m28	0	Double
MBD2_siRNA	Generic	m29	1	Boolean
shMyb501	Generic	m30	1	Boolean
shBCL11A	Generic	m31	1	Boolean
CHD4_siRNA	Generic	m32	1	Boolean
BCL11A_siRNA	Generic	m33	1	Boolean
FOG1_siRNA	Generic	m34	1	Boolean
HDAC1/2_siRNA	Generic	m35	1	Boolean

Table 2. Processes in the HFPN model of human fetal-to-adult hemoglobin switching.

Biological phenomenon	Process	Type	Rate	Delay
Transcription of KLF-1mRNA	T1	Continuous	m1*0.1	0
Translation of KLF-1	T2	Continuous	m2*0.1	0
Transcription of BCL11AmRNA	T3	Continuous	m3*1	0
Translation of BCL11A	T4	Continuous	m4*0.1	0
Transcription of HDAC1/2mRNA	T5	Continuous	1	0
Translation of HDAC1/2	T6	Continuous	m6*0.1	0
Transcription of MDB2mRNA	T7	Continuous	1	0
Translation of MDB2	T8	Continuous	m7*0.1	0
Transcription of CHD4mRNA	T9	Continuous	1	0
Translation of CHD4	T10	Continuous	m10*0.1	0
Binding of HDAC1/2, MDB2 and CHD4	T11	Continuous	m7*m9*m11*0.1	0
Binding of NuRD with BCL11A	T12	Continuous	m5*m12*0.1	0
Transcription of GATA1mRNA	T13	Continuous	1	0
Translation of GATA1	T14	Continuous	m14*0.1	0
Transcription of FOG1mRNA	T15	Continuous	1	0
Translation of FOG1	T16	Continuous	m16*0.1	0
Transcription of SOX6mRNA	T17	Continuous	1	0
Translation of SOX6	T18	Continuous	m18*0.1	0
Binding of GATA1, FOG1 and SOX6	T19	Continuous	m15*m17*m19*0.1	0
Binding of ETF with BCL11A_NuRD	T20	Continuous	m13*m20*0.1	0
Binding of BCL11A_NuRD_ETF with γ-globin gene	T21	Continuous	m21*m23*0.1	0
Activation of γ-globin gene	T22	Continuous	0.01	0
Transcription of γ-globin mRNA	T23	Continuous	m23*0.1	0
Translation of HbF	T24	Continuous	m24*0.1	0
Activation of β-globin mRNA by KLF-1	T25	Continuous	m3*0.002	35
Activation of β-globin mRNA by GATA1	T26	Continuous	m15*0.002	35
Activation of β-globin mRNA by FOG1	T27	Continuous	m17*0.002	35
Translation of HbA	T28	Continuous	m27*0.1	0
Binding of siMBD2 to MBD2mRNA	T29	Continuous	m8*0.76	0

Binding of shMyb501 to KLF-1 mRNA	T30	Continuous	m2*0.58	0
Binding of shBCL11A to KLF-1 mRNA	T31	Continuous	m2*0.02	0
Binding of shBCL11A to BCL11A mRNA	T32	Continuous	m4*1.5	0
Binding of CHD4siRNA to KLF-1 mRNA	T33	Continuous	m2*0.45	0
Binding of CHD4siRNA to BCL11A mRNA	T34	Continuous	m4*0.21	0
Binding of BCL11AsiRNA to BCL11AmRNA	T35	Continuous	m4*4	0
Binding of FOG1siRNA to FOG1mRNA	T36	Continuous	m16*1	0
Binding of HDAC1/2siRNA to HDAC1/2mRNA	T37	Continuous	m6*1	0

Table 3. Degradations in the HFPN model of human fetal-to-adult hemoglobin switching.

Phenomenon	Process	Type	Rate
mRNA degradation	d1 – d10	continuous	mi*0.1
Protein degradation	d11 – d24	continuous	mi*0.01

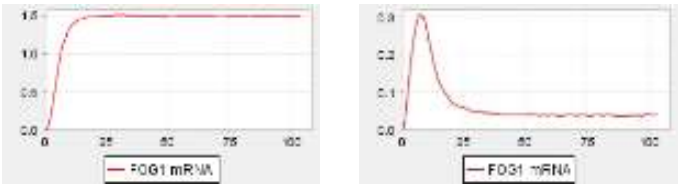
Table 4. Connectors in the HFPN model of human fetal-to-adult hemoglobin switching.

Connector	Firing Style	Firing script	Type
c1 – c68	Threshold	0	Input process
C69 – c98	Threshold	0	Output process
C99 – c113	Threshold	0	Input inhibitor
c114	Threshold	0	Input association

To study human γ-globin gene regulation, experiments were carried out in chemical inducer dimerization (CID) - dependent mouse bone marrow cells carrying β-globin yeast artificial chromosome (β-YAC). It is broadly known that human γ-globin gene is repressed in these adult phenotype erythroid cells [6]. It was also observed that in CID cells siMBD2 treatment reduces expression of MBD2 by approximately 80%, derepressing γ-globin expression [13]. Simulation results for expression of MBD2 mRNA are shown in Fig.7(a) for an untreated cell and in Fig.7(b) for a cell treated with siMBD2 technique. By carefully calibrating the rate of binding between siMBD2 and MBD2 mRNA we achieved 5-fold decrease from 5 down to 1 for MBD2 mRNA concentration, which is a good fit to corresponding wet lab observation.

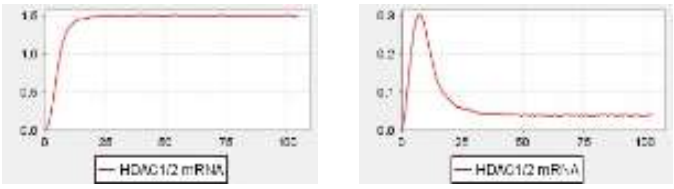
Myb has been recognized as a critical upstream regulator of KLF1 and BCL11A gene expression [24]. As determined by qRT-PCR following transduction of murine erythroleukemic (MEL) cells with virally encoded shRNA, KLF1 and BCL11A mRNA expression levels reduced following Myb knock down. Two shMyb constructs, shMyb500 and shMyb501, were tested for suppressing KLF1 and BCL11A. It was observed that shMyb501, the most efficient construct, reduces KLF1 and BCL11A mRNA levels by 75% and 76%, respectively. [24]. We validated our model in accordance with shMyb501 treatment. Simulation results for KLF1 in Fig.6(a-b) and for BCL11A in Fig.8(a-b) are in agreement with experimental results discussed above, demonstrating 4-fold decrease from 1.25 down to 0.31 for KLF1 and 4.2-fold decrease from 0.14 down to 0.033 for BCL11A.

It was also reported that BCL11A knock down by shBCL11A in MEL cells respectively suppresses KLF1 by 10% and BCL11A by 82% [24]. Simulation results for KLF1 are represented in Fig.6(a) for untreated cell and in Fig.6(c) for cells treated with shBCL11A. Comparing simulation results we conclude that BCL11A reduces KLF1 mRNA level by 1.1-fold from 1.25 down to 1.13. Similarly, comparison of simulation results for BCL11A in untreated cell (Fig.8(a)) with those in shBCL11A treated cells (Fig.8(c)) shows suppression of BCL11A expression by 5.6-fold from 0.14 down to 0.0252. Simulation results for KLF1 and BCL11A confirm the experimental data reported in [24].



(a) (b)

Fig 4: Simulation results for expression of FOG1 mRNA in (a) an untreated cell; (b) a cell treated with our RNAi-mediated strategy. Simulation results for potential RNAi treatment demonstrates decrease of FOG1 mRNA level by 37.5-fold from 1.5 to 0.04, which is equivalent to its suppression by approximately 97% over an untreated one.



(a) (b)

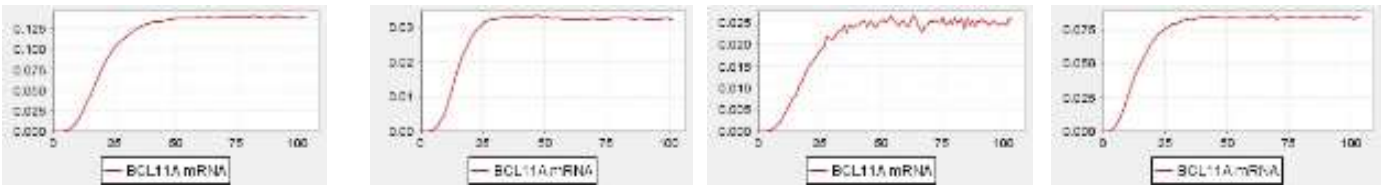
Fig.5: Simulation results for expression of HDAC1/2 mRNA in (a) an untreated cell; (b) a cell treated with our RNAi mediated strategy. Simulation results for potential RNAi treatment demonstrates decrease of HDAC1/2 mRNA level by 37.5-fold from 1.5 to 0.04, which is equivalent to its suppression by approximately 97% over an untreated cell.

(a) (b) (c) (d)

Fig.6: Simulation results for expression of KLF-1 mRNA in (a) an untreated cell; a cell treated (b) with shMyb501; (c) with shBCL11A; and (d) with siCHD4. Simulation results for treatment with shMyb501, shBCL11A and siCHD4 demonstrate suppression of KLF-1 mRNA by 75%, 10% and 70%, respectively. In the case of treatment with shMyb501 KLF-1 mRNA level is decreased from 1.25 of untreated case down to 0.31 by 4-fold, with shBCL11A from 1.25 to 1.13 by 1.1-fold, and with siCHD4 from 1.25 to 0.375 by 3.3-fold. All these results are in strong agreement with web lab observations.

(a) (b)

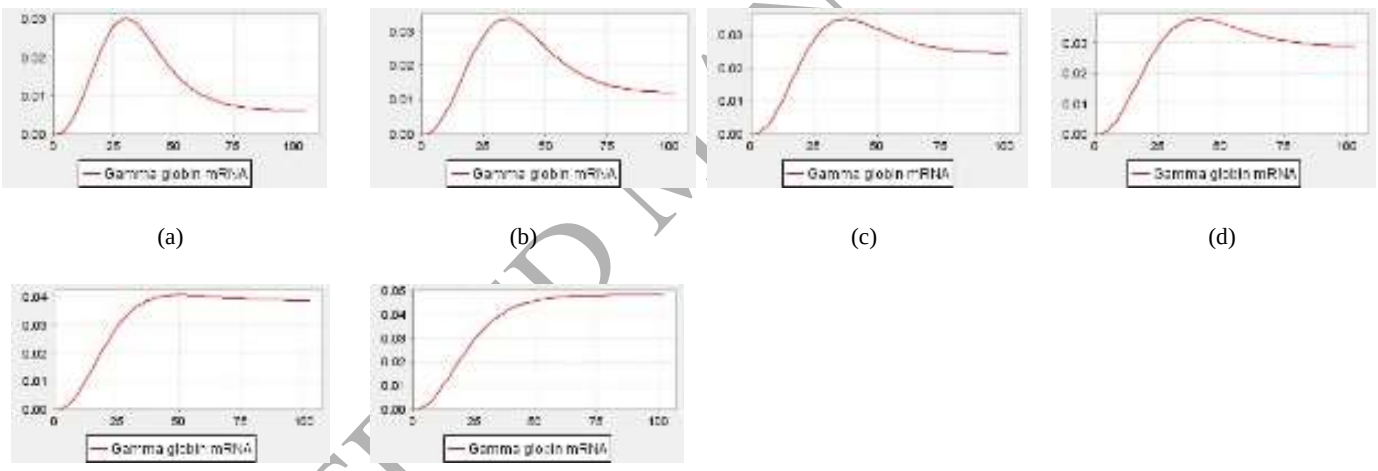
Fig.7: Simulation results for expression of MBD2 mRNA in (a) an untreated cell; (b) a cell treated with siMBD2 technique. Treatment with siRNA technique suppresses MBD2 mRNA expression by approximately 80% over the untreated control. Simulation results match with this observation in sense that MBD2 mRNA level is decreased by 5-fold from 5 to 1.



(a) (b) (c) (d)

(e)

Fig.8: Simulation results for expression of BCL11A mRNA in (a) an untreated cell; a cell treated with (b) shMyb501; (c) shBCL11A; (d) siCHD4; and (e) our RNAi-mediated strategy. Treatment with shMyb501, shBCL11A and siCHD4 respectively reduces BCL11A mRNA concentration by 76%, 82% and 40% over the untreated control. Simulation results with shMyb501 suppresses BCL11A mRNA expression from 0.14 down to 0.033 by 4.2-fold, with shBCL11A from 0.14 down to 0.0252 by 5.6-fold, with siCHD4 from 0.14 down to 0.084 by 1.7-fold, with our RNAi-mediated strategy from 0.14 down to 0.0125 by 11.2-fold.



(a) (b) (c) (d)

(e) (f)

Fig.9: Simulation results for expression of γ-globin mRNA in (a) an untreated cell; a cell treated with (b) siMBD2; (c) shMyb501; (d) shBCL11A; (e) siCHD4; and (f) RNAi technique suppressing BCL11A, FOG1 and HDAC1/2. Simulation results for siMBD2, shMyb501, shBCL11A, siCHD4 and our proposed RNAi strategy respectively show 1.9-, 3.4-, 4-, 5- and 6-fold increase of γ-globin mRNA level over the untreated control.

CHD4 binds directly to and positively regulates both the KLF1 and BCL11A (see Fig.1) [3]. CHD4 also represses γ-globin gene indirectly by binding to and positively regulating expression of BCL11A. As it is reported in [3] siRNA-mediated knockdown of CHD4 in CID cells suppresses KLF1 by 70% and BCL11A by 40%. By comparing simulation results for untreated cell in Fig.6(a) and siRNA-mediated cell in Fig.6(d) we find that CHD4 knock down suppresses KLF1 mRNA concentration by 3.3-fold from 1.25 down to 0.375. Likewise, comparison of simulation results for untreated cell in Fig.8(a) with siRNA treated one in Fig.8(d) demonstrates decrease by 1.7-fold from 0.14 down to 0.084 in BCL11A mRNA level. Both cases provide exact validation between experimental and numerical data.

We have also found that our RNAi-mediated strategy decreases BCL11A, mRNA level by 11-fold from 0.14 to 0.0125 (see Fig.8(a) and Fig.8(e)), FOG1 mRNA by 37.5-fold from 1.5 to 0.04 (see Fig.4(a) and Fig.4(b)), HDAC1/2 mRNA level by 37.5-fold from 1.5 to 0.04 (see Fig.5(a) and Fig.5(b)).

3.3. Identifying optimal targets for gene therapy of β-globin disorders

Several researchers have studied regulators of fetal-to-adult hemoglobin switching network as potential targets for siRNA- and shRNA-mediated reactivation of γ-globin gene. To the best of authors' knowledge all such targets with available qPCR data are collected under strategies (1)-(4). The question arises as to whether there exists a target-based gene therapeutic strategy that leads to more γ-globin production. To answer the question, we have performed computer simulations on various combinations of major regulators and picked up the one that leads to significant increase of γ-globin gene expression. Computer experiments for siMBD2 in Fig.9(b) show increase of γ-globin gene expression from 0.008 to 0.015, which is equivalent to 1.9-fold increase over the untreated control, for shMyb501 in Fig.9(c) from 0.008 to 0.027 by 3.4-fold, for shBCL11A in Fig.9(d) from 0.008 to 0.032 by 4-fold, for siCHD4 in Fig.9(e) from 0.008 to 0.04 by 5-fold and finally for our RNAi strategy in Fig.9(f) from 0.008 to 0.048 by 6-fold increase over the untreated control. Simulation results for strategies (1)–(5) reveal that our strategy is the optimal among five strategies detailed in the present research as it leads to maximum increase of the γ-globin mRNA level.

4. Discussion and conclusions

The present research uses quantitative modeling with HFPN for identifying the optimal molecular targets to ameliorate the severity of β-globin diseases, and thereby promotes this innovation to the benefit of both reverse pharmacology and quantitative modeling with HFPN.

The present research needs to be assessed from the perspective of the rationalistic approach as we focus on determining optimal targets for RNAi-mediated mechanism leading to the maximum γ-globin gene induction. In the meantime, we try to shed light on how quantitative modeling with HFPN can be used for identifying targets for RNAi-mediated therapy without diving deep into details as how this can be achieved by pharmacological point of view. Although it is not within the scope of this paper, as future prospects, we are sure that a more detailed discussion of biological consequences should be considered in collaboration with a pharmacogenetics group. It should also be noticed that the present approach can be successfully used for identifying optimal RNAi-mediated mechanism not only for amelioration of severity of β-globin disorders but also for therapy of other diseases.

By developing deterministic quantitative model, we tried to reproduce underlying biological context based on rigorous review of biological literature available to date. This is the way how it is done in similar research works by many researchers some of which are cited in this paper. So we hope our model is trustful enough as we tried to integrate all known molecular interactions between the biological components of fetal-to-adult hemoglobin switching network. It is often that because simulations are quite expensive for large stochastic systems in such models one often wishes to know whether or not the stochastic system can be approximated by a deterministic one when underlying biological network is sufficiently large. In a sense, instead of creating a stochastic model and then reducing the model to a deterministic one we develop a deterministic model from scratch. However, we do understand that influence of noises in biological systems is important. As a further work we are planning to integrate stochastic parameters to the model to see the effect of the noises to underlying biological system.

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