Network Modeling Analysis in Health Informatics and Bioinformatics (2018) 7:14
https://doi.org/10.1007/s13721-018-0175-z

ORIGINAL ARTICLE

Network-based approach to understand dynamic behaviour of Wnt signaling pathway regulatory elements in colorectal cancer

Ankita Shukla[1] · **Tiratha Raj Singh**[1]

Received: 22 January 2018 / Revised: 29 August 2018 / Accepted: 1 September 2018 / Published online: 7 September 2018

Abstract

Systems biology helps to understand the intricate biological processes through the regulatory and metabolic mechanism. Modeling and simulation are the computational approaches to encounter the physiological and disease processes by means of an artificial environment that precisely mimics the conditions inside the cell. A large proportion of colorectal cancers (CRC) display mutational inactivation of the variety of pathways and Wnt-signaling is thought to be one of the major contributors from all the pathways that show progression towards CRC. In our study, we have performed a computational analysis to envisage the role of candidate genes for the Wnt signaling pathway. Quantitative simulations have been performed for the colorectal carcinoma. In addition, network motifs detection was performed so as to decipher the role of crucial components in the pathophysiology of CRC. Based on the standard statistical parameters such as Z score, p value and significance profile candidate genes were recovered from the Wnt pathway. The proposed method revealed statistical significance of five key genes i.e. *Axin, APC, β-catenin, Lef1*, and *Myc* reflecting their importance to study disease condition. These genes could prove to be efficient markers for the disease diagnosis and also provide a way to solve the mystery behind the aberrant regulation of Wnt signaling in CRC.

Keywords Colorectal cancer · Modeling · Simulation · Signaling pathway · Network motifs · Carcinogenesis

1 Introduction

Colorectal cancer (CRC) is the third most commonly diagnosed malignancy and the fourth leading cause of cancer related deaths in the world (Arnold et al. 2016). However, the disease is most prominent in developed countries than the developing or the underdeveloped ones (Tariq and Ghias 2016). In general incidences, where the mortality rate is higher in men than in women shows high risk of males towards cancerous condition (Hisamuddin and Yang 2006). Along with the genetic conditions, bad lifestyle, and aging are the contributing factors towards CRC incidences and metastasis (Grady and Markowitz 2002; Haggar and Boushey 2009). There are many factors such as chemical, environmental, and lifestyle that are responsible for the CRC initiation and its progression. Although this cancer occurs at the later ages, current records suggest incidences among the young population. The reasons being adapting unhealthy livelihood such as long duration of physical inactiveness, obesity, smoking, red meat intake and alcohol addiction (Li and Martin 2016; Markowitz and Bertagnolli 2009; Martin 2003).

DNA repair mechanisms play a key role in repairing the damages inflicted to the DNA and thereby regulates the cancer progression (Dietlein et al. 2014; Gavande et al. 2016; Markowitz and Bertagnolli 2009; Shukla et al. 2015, 2016, 2018). It has been found from the studies that the mismatch repair (MMR) mechanisms act as a common etiologic factor in the CRC (Peltomaki 2001). Cell signaling pathways do not function alone but are interconnected by some means and form complex signaling networks. Different types of growth factor receptors, cell–matrix, and cell–cell interactions convey the signals inside the cell where they are processed. The signals that are received regulate diverse processes, such as protein synthesis, cell growth, motility, architecture, polarity, differentiation, and programmed cell death (Tariq and Ghias 2016). The same signaling molecules co-operate diverse processes within different signaling complexes or at different

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s13721-018-0175-z) contains supplementary material, which is available to authorized users.

✉ Tiratha Raj Singh
tiratharaj@gmail.com

[1] Department of Biotechnology and Bioinformatics, Jaypee University of Information Technology (JUIT), Waknaghat, Solan, HP 173234, India

intracellular locations. Depending upon the cellular milieu the activated signaling molecule may produce distinctive consequences. Understanding the role of these complex signaling networks in cancer cells is cognitive to the scientific community. Here, we targeted Wnt signaling pathway as many studies have shown its key role in maintenance of the proper architecture of the intestinal epithelium (Schneikert and Behrens 2007). Furthermore, the normal proliferation of the precursor cells depends on permanent activation of this pathway. Wnt activation stimulates via the accumulation of nuclear *β-catenin* which are formed at the bottom of normal crypts. The crypts in the intestine get washed off due to the transgenic expression of the Wnt inhibitor Dickkopf1 (*Dkk1*). In comparison, stimulation of the pathway through mutation of the *APC* (which acts as a negative regulator) provokes the hyperproliferation of the epithelium. So, by keeping this in mind we have worked on the Wnt model (Fig. 1) and its simulation (Chelliah et al. 2013; Sivakumar et al. 2011). Behavior analysis has been done by performing quantitative simulations for all the candidate genes for the pathway of interest (MathWorks 2012).

Along with the dynamic behavior analysis an endeavor was made to identify vital components of the pathway wherein network motifs were identified which supported the role of crucial components in the pathway analysis. Network motifs are made up of a small set of recurring regulation patterns that serve as basic building blocks of transcription networks (Alon 2007). The network modules and motifs play key role in detection and analysis of explicit patterns in biological networks and capitulate significant insights for better understanding of complex biological processes involved in intricate human diseases (Barabási et al. 2011). Computational and statistical methods were applied for the detection of significant network motifs and functional estimation measures were implicated to reduce the complexity of the network and to find the best appropriate candidates for their further biological validation proposals.

In some of the earlier studies on Wnt signaling, Lee et al. studied the role of *Axin* and *APC* in the formation of degradation complexes along with the dependence of *Axin* degradation on *APC* (Lee et al. 2003). In Kruger and Heinrich study robustness of the signaling pathway with respect to the parameter fluctuations were considered (2004). Cho et al. studied effect of the mutations of the *APC, Axin*, and *β-catenin* on the signaling state, and above all explained the preference of *APC* mutations (to the other pathway components) for the occurrence of CRC (2006). Van Leeuwen et al. showed that it is the amount of *APC* that decides the fate of Wnt signaling for the normal or diseased phenotype (CRC) (2007). Goldbeter and Pourquié studied the oscillatory behavior of the Wnt pathway which showed the negative feedback exerted by axin through the formation of the destruction complex on the degradation of the *β-catenin* which can produce oscillations in the pathway (2008). In Mirams et al. study pathway has been simplified to

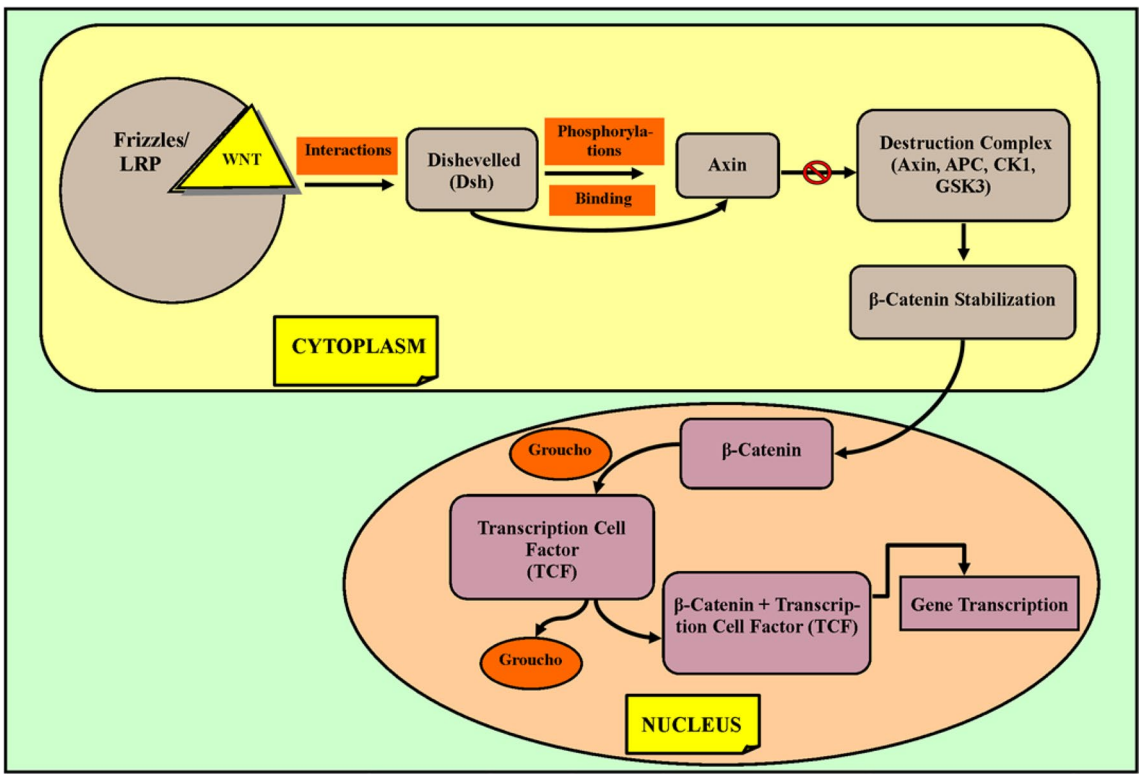

Fig. 1 Overview of Wnt canonical signaling pathway mechanism

get the crucial details at distinct timescales, the fastest timescale is related to the action of the destruction complex for the *β-catenin*; the intermediate timescale captures the impressions made by the dominant reactions that regulate the level of destruction complex with the removal of the axin under the influence of the *Wnt* and Dishevelled (Dsh); and the slowest timescale marks the changes in the *β-catenin* level (2010). The study performed by MacLean et al. included the single model wherein non-dimensionalization was applied for analyzing the steady state behavior under asymptotic conditions (2016). The study performed by the Sivakumar et al. was conducted on the neural stem cell differentiation by considering notch, shh, Wnt, and EGF pathways in presence of the targeted drugs (2011). The study performed by the Kestler and Kuhl (2008) applied the deterministic approaches for the conditions where Wnt proteins activate a complex set of intracellular signaling network rather than a individual pathway. In the method given by the Saha et al. (2012) an approach was followed to identify the novel members of the Wnt regulatory network as well as the subnetworks of the larger Wnt signaling network that are active in different biological contexts. All of the above-mentioned studies analyzed the individual effect of the component, however, in our studies we tried to relate the key components of the pathway to the various complexes and with varying concentrations in a different environment.

In case of the Wnt signaling pathway, our analysis provides a basis for understanding the behavioral dynamics of genes and proteins while performing the quantitative perturbation. The quantitative approach used in this paper provides good information for understanding model behavioral dynamics which we hope will prove useful to the researchers working in the field of systems biology. The simulations performed by considering all set of entities and rate kinetics helped to identify the rhythmic patterns of entities for their non-steady to steady state. While looking at the behavior of all entities, few entities were selected based upon their dynamical behavior towards the overall pathway. Therefore, to focus on these specific entities, separate simulations were performed. This study describes the phenomenon of activation and suppression points of the proteins (captured in time frames) in regulating the carcinogenesis. This work provides a basis for understanding the dynamics involved in the pathway via key genes (tumor suppressor, oncogene) that will be helpful to develop the pharmacological strategies to regulate the pathway through therapeutic interventions.

2 Materials and methods

The quantitative simulation study was performed by considering the Sivakumar et al. model as a reference to study the biological signaling processes. The simulations were performed through MATLAB toolbox, SimBiology that provides an application and programmatic tools to model, simulate, and analyze dynamic systems focusing on systems biology applications. The workflow is given in Fig. 2. For behavior analysis species are set to different concentrations with respect to the time period. The studies majorly focused on the canonical Wnt signaling (i.e. when Wnt is present as a stimulus), which encompass cellular responses to Wnt ligand-mediated by *β-catenin* (MacLean et al. 2016). The reaction mechanism follows mass action kinetics, which states that a reaction proceeds at a rate proportional to the product of its reactants. Mathematical representation of the Fig. 1 in the form of ODEs that specifies the model are given as follows:

$$Dsh_i' = -k1Dsh_i + k2Dsh_a, \tag{1.1}$$

$$Dsh_a' = k1Dsh_i - k2Dsh_a, \tag{1.2}$$

$$\begin{aligned} Dest\ Complex_a' = {} & k3Dest\ Complex_i - k4Dest\ Complex_a \\ & - k10\beta - catenin \cdot Dest\ Complex_a \\ & + k11(Complex)\beta - catenin \cdot Dest\ Complex \\ & + k13(Complex)\beta - catenin \\ & \cdot Dest\ Complex(proteasomal\ degradation), \end{aligned} \tag{1.3}$$

$$\begin{aligned} Dest\ Complex_i' = {} & k6GSK3 \cdot (Complex)Axin \cdot APC \\ & - k5Dsh_a \cdot Dest\ Complex_i \\ & - k3Dest\ Complex_i + k4 \\ & Dest\ Complex_a - k7Dest - Complex_i, \end{aligned} \tag{1.4}$$

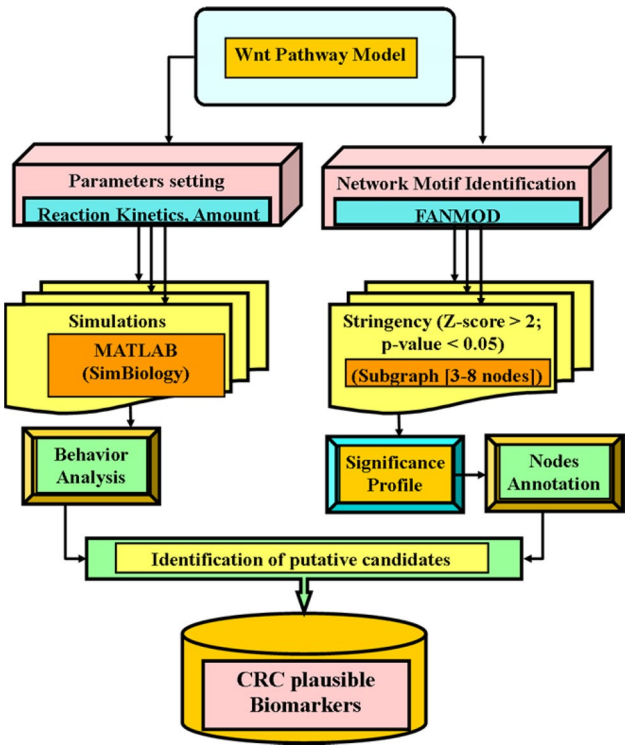

Fig. 2 Strategy followed for simulation and analysis

$$GSK3' = k5Dsh_a \cdot Dest\ Complex_i - k6GSK3 \cdot (Complex)Axin \cdot APC + k7Dest\ Complex_i, \quad (1.5)$$

$$(Complex)Axin \cdot APC' = k5Dsha \cdot Dest\ Complex_i - k6GSK3 \cdot (Complex)Axin \cdot APC + k7Dest\ Complex_i + k8Axin \cdot APC - k9(Complex)Axin \cdot APC, \quad (1.6)$$

$$APC' = -k8Axin \cdot APC + k9(Complex)Axin \cdot APC - k21\beta\ catenin \cdot APC + k22(Complex)\beta - catenin \cdot APC, \quad (1.7)$$

$$(Complex)\beta\ catenin \cdot Dest\ Complex' = k10\beta\ catenin \cdot Dest\ Complex_a - k11(Complex)\beta - catenin \cdot Dest\ Complex - k12(Complex)\beta - catenin \cdot Dest\ Complex, \quad (1.8)$$

$$(Complex)\beta\ catenin \cdot Dest\ Complex(proteasomal\ degradation)' = k12(Complex)\beta\ catenin \cdot Dest\ Complex - k13(Complex)\beta\ catenin \cdot Dest\ Complex(proteasomal\ degradation), \quad (1.9)$$

$$\beta\ catenin(proteasomal\ degradation)' = k13(Complex)\beta\ catenin \cdot Dest\ Complex(proteasomal\ degradation) - k14\beta\ catenin(proteasomal\ degradation), \quad (1.10)$$

$$\beta\ catenin' = -k10\beta\ catenin \cdot Dest\ Complex_a + k11(Complex)\beta\ catenin \cdot Dest\ Complex + k15 - k16\beta\ catenin - k19\beta\ catenin \cdot TCF + k20(Complex)\beta\ catenin \cdot TCF - k21\beta\ catenin \cdot APC + k22(Complex)\beta\ catenin \cdot APC, \quad (1.11)$$

$$Axin' = -k8Axin \cdot APC + k9(Complex)Axin \cdot APC + k17 - k18Axin, \quad (1.12)$$

$$TCF' = -k19\beta\ catenin \cdot TCF + k20(Complex)\beta\ catenin \cdot TCF, \quad (1.13)$$

$$(Complex)\beta\ catenin \cdot TCF' = k19\beta\ catenin \cdot TCF - k20(Complex)\beta\ catenin \cdot TCF, \quad (1.14)$$

$$(Complex)\beta\ catenin \cdot APC' = k21\beta\ catenin \cdot APC - k22(Complex)\beta\ catenin \cdot APC, \quad (1.15)$$

where primes denote the differentiation with respect to time and k_n ($n = 1, 2, ..., 22$) denotes the non-negative rate constants. This specifies the reaction kinetics, and thus helps to study the dynamic behavior of the biochemical pathway. The ODE solver was used to perform the simulations for all these reactions and their respective parameters. The simulations were compiled for all the candidate genes by repeating the experiments for various sets of parameters (Supplementary Fig. 1). The solver considered for the run is Ode45 (Dormand–Prince), which is an explicit method for solving ordinary differential equations, and it also minimizes the error rate and, therefore, considered more suitable when the higher-order solution is used to continue the integration (Dormand and Prince 1980). Diverse parameters were selected based upon the standards given in the literature (Supplementary Table 1) (Hochman et al. 2017; Tan et al. 2012; Young et al. 2018). The simulation time is set to the stop time of 3 s and absolute tolerance is set to 1.0E − 6 with relative tolerance of 0.001 for first run of the simulation. By setting the above parameters simulations were performed for the entire components of the model. The concentrations mentioned in the adapted model for the Wnt pathway are extracted through the SBMLsqueezer; a CellDesigner plugin which generates kinetic rate equations for the biochemical network (Drager et al. 2008). For each reaction, the kinetic equation is derived from the stoichiometry, the regulatory mechanisms and the participating species (proteins, simple molecules). The rate laws are generated by considering each reaction for all participating reactants, products, and the regulators. To understand the signaling process we have considered the set of all components of the Wnt pathway to capture the effect of one over the other. Since the overall signaling process revolves around the *β-catenin* level and the destruction complexes that make a check regarding the overproduction of the *β-catenin* that in case of deregulation cause the transcription of the targeted genes thus leading its way towards cancer.

The network motif study have been done by considering the complete Wnt pathway retrieved from the KEGG pathway database (Kanehisa 2002). For detection of the network motifs, FANMOD was used with statistical parameters such as Z value > 2 and p value < 0.05 to generate subgraphs of 3–8 nodes size (Supplementary Table 2) (Wernicke and Rasche 2006). After then significance profile (SP) has been computed so as to assess the statistical implication of the generated motifs. SP generates normalized Z score values for individual network motif that is given as:

$$\mathrm{SP}(m_i) = \frac{Z(m_i)}{\sqrt{\sum_{i=1}^{n} Z(m_i)^2}},$$

where m_i represents network motif and $Z(m_i)$ the Z score for individual network motif. The SP method thus provides the subgraphs with statistical significance (Supplementary Table 2). Annotation has been performed for the ones that

were found to occur at higher frequency rate even after the logarithmic conversion.

3 Results and discussion

3.1 Quantitative simulations (the behavioural study)

The simulations were performed at different time periods by considering different entities; for which we found some interesting behavior that was not targeted in the studies performed earlier. Initiating the simulation by taking into account the β-catenin (plasma membrane) having concentration of 5-units, the complex-1 (*APC, β-catenin, GSK3β, Axin, PP2A, Diversin, CK1*) having concentration of 5-units and the complex-2 (*APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β*) was kept at 0-units (Table 1). Some interesting behaviors were observed as shown in Fig. 3. The result has shown that even though there is high amount of *β-catenin* (a subunit of the cadherin protein complex and has a role in regulation and coordination of cell–cell adhesion and gene transcription) but as long as it is captured by the destruction complex, *β-catenin* slows down its activity and its concentrations diminishes from its initial amount of 5-units to 0.2-units. Thus, illustrating the fact that the high concentration of the *β-catenin* will not work until the destruction complex is there and it will regulate the level of *β-catenin* irrespective of its own higher or lower concentrations as shown in graph at a time it is having concentration of 5-units and 0-units. Complex-1 and *β-catenin* have same behavior while complex 2 is showing the opposite behavior. However, all three reaching at steady state at almost same time and this reflects that initial few (3–4) s are crucial for their activity to finally reach and follow the steady state (Fig. 3a).

The next iteration was performed by considering the same species as in previous simulation but this time all were set to the equal amount; this time we found interesting behavior as both the complexes are overruling, and thus not allowing the *β-catenin* to overcome the signaling process, this states that the amount of these two complexes are crucial for controlling the *β-catenin* level (Fig. 3b). Previous two simulations were indicative of the initial activity at 3–4 s, therefore, to look for this effect next iterations were performed for another 3 s by considering the species [Complex (*Wnt/Frizzled*), *β-catenin*, and Complex (*TCF, Smad4, β-catenin*)]. In the next simulation (Fig. 4a), we found that once the Wnt binds to the frizzled receptor the signaling starts and the *β-catenin* level in the plasma membrane downfall and rises up to certain extent (0.2 s) in the nucleus this initiate the transcription (when binds to the *TCF*) by activating the tumor genes that associates a step towards

Table 1 Simulation parameters for all the analyzed species with varied concentrations

Type	Name	Location	Concentrations	Solver	Simulation time	Absolute tolerance	Relative tolerance
Species	β-Catenin	Plasma membrane	5				
Complex	APC, β-Catenin, GSK3β, Axin, PP2A, Diversin, CK1	Plasma membrane	5				
Complex	APC, Axin, PP2A, Diversin, CK1, β-Catenin, β-TrCP, GSK3β	Plasma membrane	0	Ode45 (Dormand–Prince)	10 s	1.0E−6	0.0010
Complex	APC, Axin, PP2A, Diversin, CK1, β-Catenin, β-TrCP, β-TrCP, GSK3β	Plasma membrane	5				
Complex	Complex_br_(Wnt/Frizzled)	Plasma membrane	0				
Species	β-Catenin	Plasma-membrane	5				
Species	β-Catenin	Nucleus	0				
Complex	TCF, Smad4, β-Catenin	Nucleus	0	Ode45 (Dormand–Prince)	3 s	1.0E−6	0.0010
Species	β-Catenin	Nucleus	0.53				
Complex	APC, Axin, PP2A, Diversin, CK1, β-Catenin, β-TrCP, GSK3β	Nucleus	0				
Species	β-Catenin	Nucleus	5				
Complex	APC, Axin, PP2A, Diversin, CK1, β-Catenin, β-TrCP, GSK3β	Nucleus	5				
Species	β-Catenin	Nucleus	6				

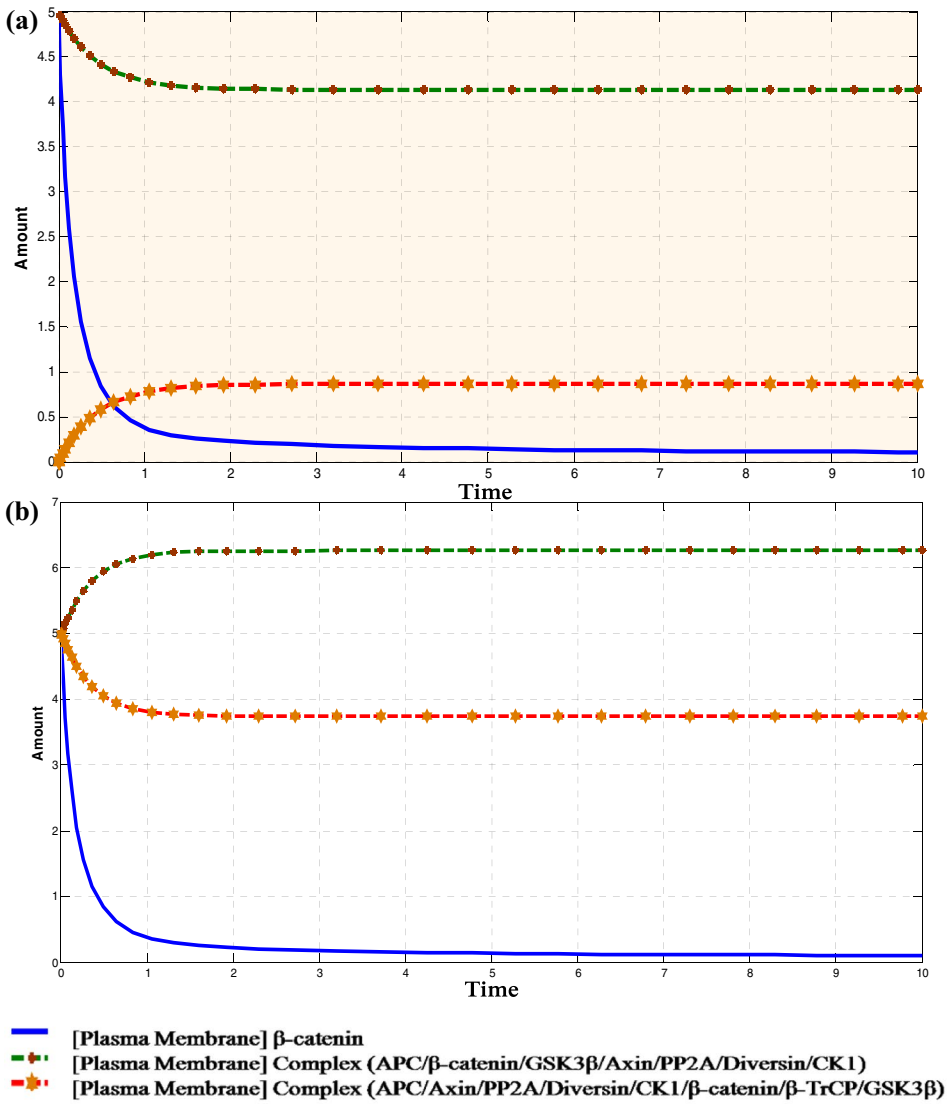

Fig. 3 Graphs representing the dynamic behavior of components in the plasma membrane. **a** Behavior of the β-catenin, Complex (APC, β-catenin, GSK3β, Axin, PP2A, Diversin, CK1), and Complex (APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β) at varied amount. **b** Behavior of the β-catenin, Complex (APC, β-catenin, GSK3β, Axin, PP2A, Diversin, CK1), and Complex (APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β) at equal amount

the cancer progression. The next iteration was performed by considering the species [*β-catenin*, and Complex (*APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β*)]. Through the simulation, we found that the complex has the strong efficiency to suppress the level of the *β-catenin* in the nucleus, and thus overcome the negative stimulation of the *β-catenin* (Fig. 4b).

The next iteration was performed by considering the species [*β-catenin*, and Complex (*APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β*)] but at varying cocentrations. Two different simulations were run; the Fig. 5a, is showing the behavior by considering the components at equal concentrations; it states the overruling behavior of the complex over the *β-catenin*. In Fig. 5b, when the concentration of the *β-catenin* is set high by one unit, it embraces the signaling process with the higher possibility of cancer progression (Fig. 5). Many other simulations were performed at varied concentrations and behavior of these regulatory elements was found similar in almost all cases (results not shown). Having detailed information regarding the parameters for all sets of entities in a pathway might further improve the analysis and investigations related to the dynamics of the pathway. However, with informed parameter guesses, simulation studies have provided good information about the system's behavior because getting knowledge related to the dynamics of the individual pathway entities is itself a cumbersome task. The powerful analytical tools highlight the successful study of the biological processes that occurs in vivo. However, such success is possible through the inexorable endeavors and the in-depth understanding of both computational methods and the biological problems of interest.

3.2 Subgraph network detection

In the subgraph network detection, the network motifs were generated by considering parameters ($Z > 2$; p value < 0.05)

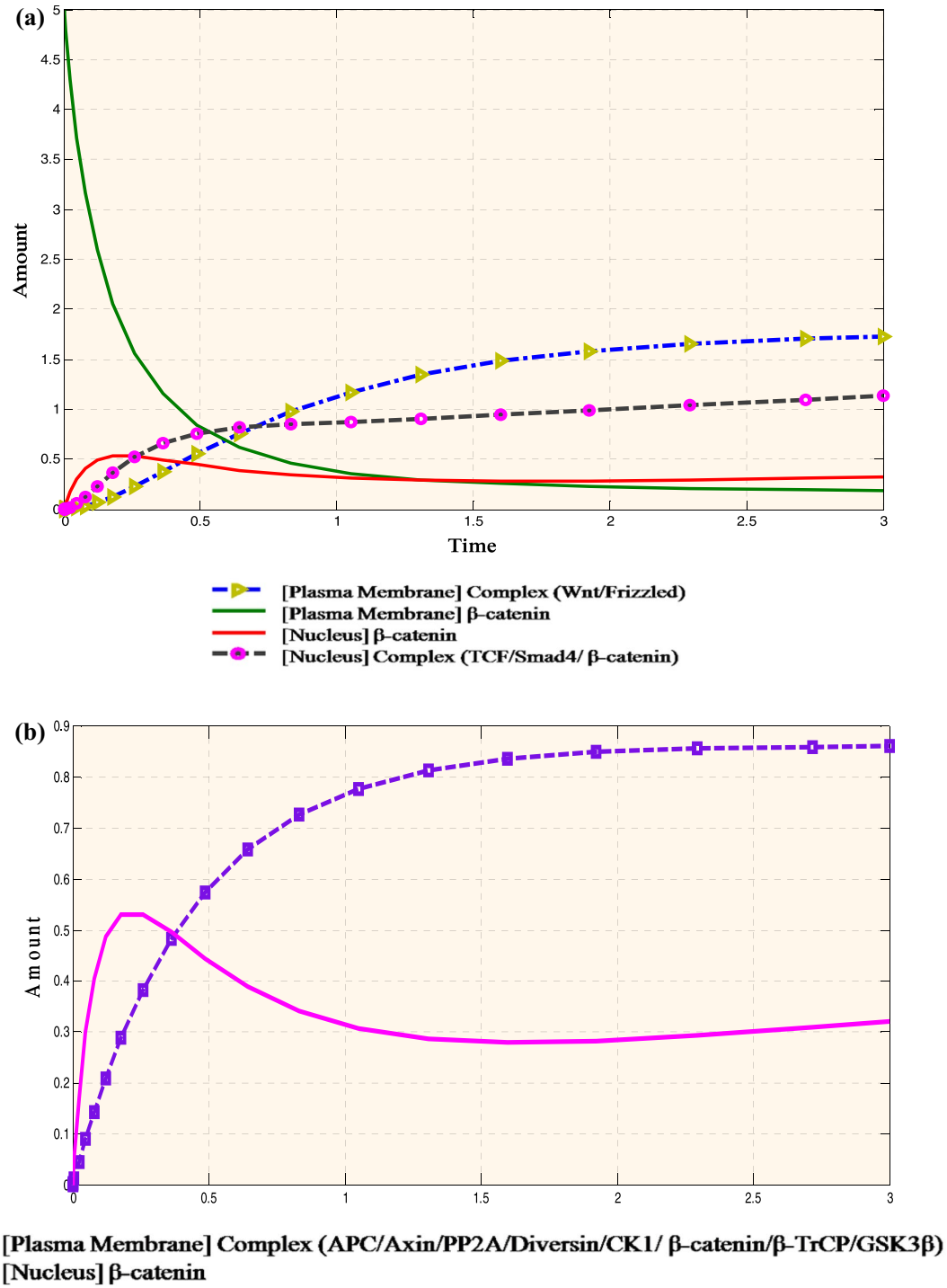

Fig. 4 Graphs representing the dynamic behavior of components in different compartments. **a** Behavior of the Complex (Wnt/Frizzled), β-catenin, and Complex (TCF, Smad4, β-catenin) and **b** behavior of the complex [i.e. β-catenin, and Complex (APC, Axin, PP2A, Diversin, CK 1, β-catenin, β-TrCP, GSK3β)] in plasma membrane vs. the β-catenin in nucleus

that produced 595 subgraphs having 3–8 nodes (Supplementary Table 2: sheet 1). SP has been calculated to normalize the Z score and to filter out the subgraphs to get the significant ones. It thus reduced the subgraphs from 595 to 64 only (Supplementary Table 2: sheet 2). The network motifs thus obtained had 4-chain motifs, single input module (SIM), multiple input module (MIM), bifan motifs, etc. that were supported by significant Z scores and p values. Other

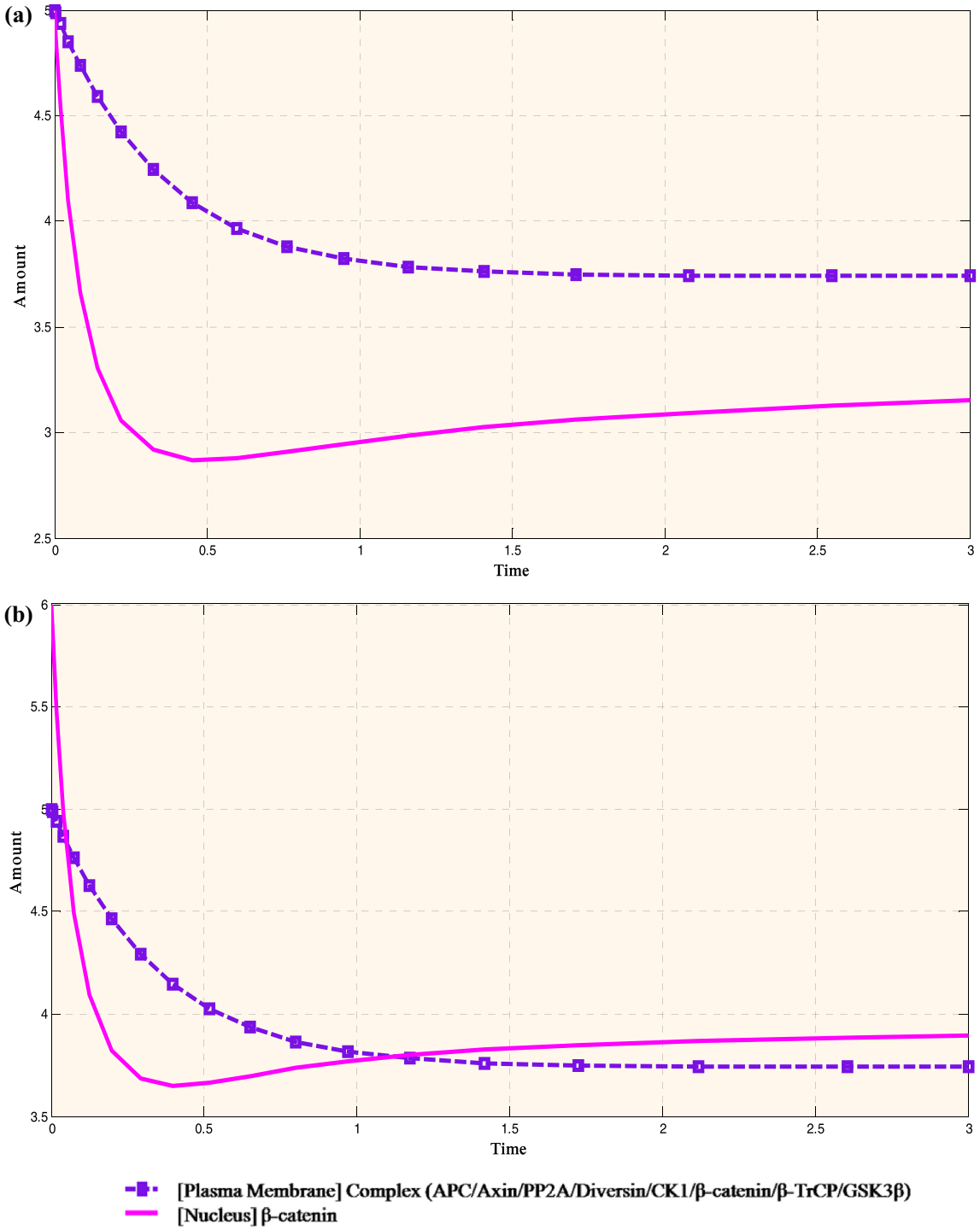

Fig. 5 Graphs representing the dynamic behavior of components in different compartments. **a** Behavior of the Complex [(APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β) and β-catenin] at equal amount. **b** Behavior of the Complex [(APC, Axin, PP2A, Diversin, CK1, β-catenin, β-TrCP, GSK3β) and β-catenin] at alternative amount

regular 4-node motifs confirmed the presence of diamond, biparallel and bifan motifs (often built by two regulatory and two regulated genes). Graph has been plotted to logarithmic scale for the significance profile generated corresponding to the particular subgraph type (Fig. 6). From the significance profile graph five subgraphs (3a, 4a, 5a, 6a, 7a) were found to be overrepresented in comparison to the other ones.

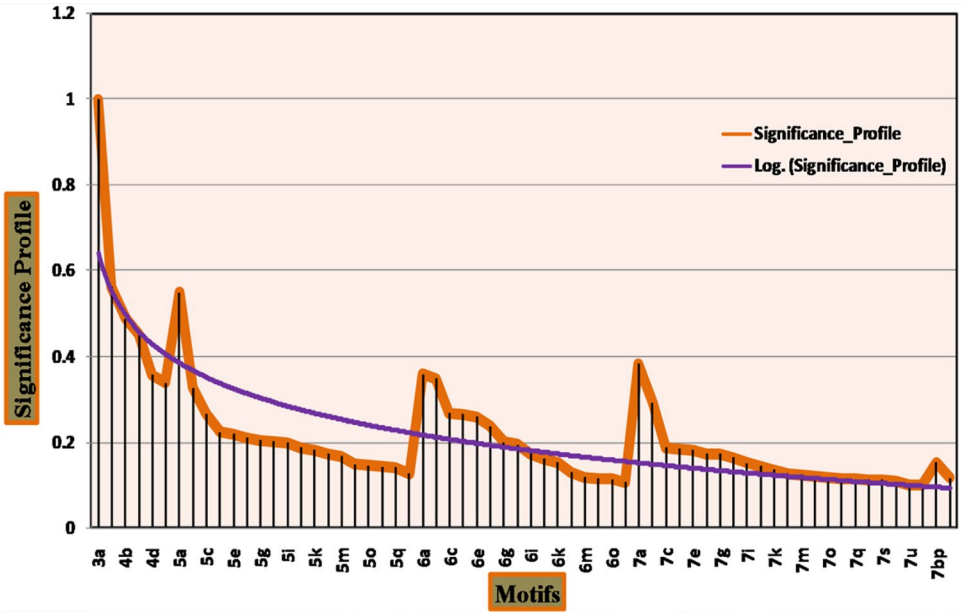

Fig. 6 Significance profile for 3–7 nodes subgraphs as network motifs. All these motifs followed a relative pattern and their significance is more when size of motif is small. It indicates their crucial and specific role at small level

The motif significance profiles illustrate that there is huge difficulty in recognizing the genes as the network starts intensifying. Therefore, the overrepresented subgraphs were annotated to find out the interacting gene partners, however, there were multiple instances for the particular subgraph type, therefore; we selected the ones with maximum frequency value of nodes that signifies the importance of these genes in the pathway (Fig. 7). The method reflected five key genes i.e. *Axin, APC, β-catenin, Lef1*, and *Myc* with high statistical significance. Some additional significant components are *MMP7, NLK* and *DVL1* which could also be important for in vitro and in vivo studies. Interaction of *MMP7* with *Lef1* represented in many motifs indicates close association of these entities. *NLK* and *DVL1* could be crucial to regulate the biological processes in the Wnt pathway as their role as potent inhibitors through *Lef1* and *GSK3β*, that indirectly negatively phosphorylate *β-catenin* (Supplementary Table 3).

MMP7 is a member of the matrix metalloproteinase (MMP) family; that is found to be overexpressed by tumor cells and has a strong tendency to enhance the tumor metastasis. Many studies indicated an enhanced level of *MMP7* mRNA, protein, specifically in human CRC liver metastasis (Zeng et al. 2002). Although according to the Zeng et al. study; *MMP7* are found to be highly regulated, and the overexpression of the *MMP7* does not always possess invasive behavior as they are mostly secreted as the latent precursors. Therefore, this behavior makes it necessary to analyze *MMP7* at different conditions in the CRC progression studies. *NLK* has been found to be an important regulator of signal transduction pathways such as Wnt and Notch, both of which play critical roles in tumorigenesis (Zhang et al. 2015). *NLK* regulates the Wnt/β-catenin signaling pathway

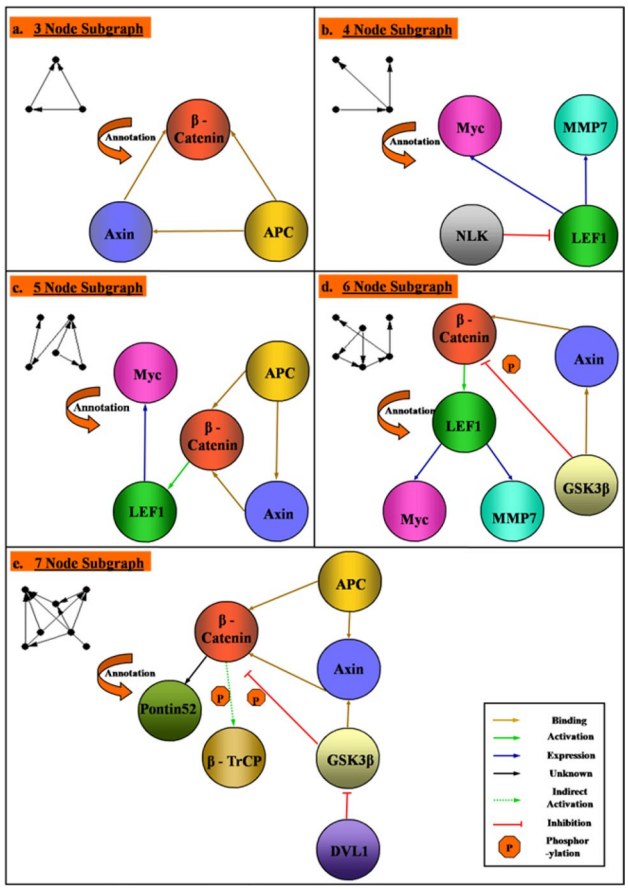

Fig. 7 Annotation of overrepresented subgraphs depicting the crucial interactions

via phosphorylating the lymphoid enhancer-binding factor 1 (*Lef1*) in neural progenitor cells (Katoh 2007). The

studies indicated the overexpression of *NLK* in advance tumors with metastasis and a high recurrence rate in CRC (Katoh 2007). The *DVL1* has been found to be overexpressed during the CRC progression which later on metastasized in liver (Huang et al. 2013). The *DVL1* overexpression may affect metastatic behavior of tumor cells in CRC patients. Therefore, these genes would prove to be a suitable marker for CRC prognosis and to analyze the metastatic conditions. Mainly, the Wnt signaling has been found to be prevalent in those CRC tumors that are being MSS (microsatellite stable) and CMS2 (consensus molecular subtype 2) conditions (Smaglo and Marshall 2013). The CRC has generally been classified into four groups each having distict expression; i.e. CMS1,CMS2, CMS3and CMS4. In our study, the data we targeted belongs to the MSS and CMS2 (canonical pathway) category; as there is no information for the loci alteration and the analysis mainly concerned to the canonical Wnt signaling, therefore, the prevalency rate for the CRC follows CMS2. The biological characterization of the type of CRC thus would prove to be helpful in terms of predicting factor for the cancer treatment procedures.

Therefore, the combination of top-down and bottom-up approaches followed not only emphasizes the major interacting partners but also uncover the ones that were not targeted yet for the abrupt regulation of Wnt signaling in CRC. Network motifs provide the way to understand the complex pathway by dividing the large complex network into the smaller network that can be evaluated through statistical means to determine its significance in biological processes. In this study, we combined the behavior analysis by considering the dynamic behavior of the individual components along with the identification of plausible biomarkers through the bottom-up approach that covers the overall aspect of the Wnt pathway in CRC. The exemplified in silico approach could be applied to any other diseases at the pathway level for identifying biomarkers and it will surely save the time of experimental biologists and to focus on the crucial components of the pathway so that the disease can be detected and possibly prevented at its early stages.

4 Conclusion

Modeling and simulation techniques provide the comprehensive method to understand the complex biological processes that are otherwise difficult to understand in vivo. Cancer is one of the complicated diseases that is difficult to eradicate only by treating it in one aspect as there are multiple possibilities for its progression. So, by considering all these aspects we have performed the simulation studies by taking care of all possible targets for CRC. This study clearly depicts the effect of the *β-catenin* and its regulation by various complexes that includes destruction complex that captures the *β-catenin* in the cytoplasm and prevent its transcription, and another one in the nucleus where it is bound to the transcription cell factor (*TrCP*) and made its progress towards CRC. Besides five key genes i.e. *Axin, APC, β-catenin, Lef1*, and *Myc* many other putative regulatory elements of the pathway such as *MMP7, NLK* and *DVL1* were computationally predicted and evaluated that could be further experimentally validated for CRC. We attempted to evaluate the current knowledge about the role of Wnt signaling pathway involved in colorectal carcinogenesis due to the target genes. Repeated patterns seems to play significant role in the biological processes; through the subgraph study we came to know the interacting partners that shares very close neighborhood but their role in CRC is still unknown. If given attention these imprints can unravel the threads of the complex network, and thus prove to be valuable insight for a disease. We hope this computational analysis will provide broad view of the disease to the biological researchers so that effective methods can be developed to eradicate this disease.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

References

Alon U (2007) Network motifs: theory and experimental approaches. Nat Rev Genet 8:450–461. https://doi.org/10.1038/nrg2102

Arnold M, Sierra MS, Laversanne M, Soerjomataram I, Jemal A, Bray F (2016) Global patterns and trends in colorectal cancer incidence and mortality. Gut. https://doi.org/10.1136/gutjnl-2015-310912

Barabási A-L, Gulbahce N, Loscalzo J (2011) Network medicine: a network-based approach to human disease. Nat Rev Genet 12:56–68

Chelliah V, Laibe C, Le Novère N (2013) BioModels database: a repository of mathematical models of biological processes. In: Encyclopedia of systems biology. Springer, New York, NY, pp 134–138

Cho KH, Baek S, Sung MH (2006) Wnt pathway mutations selected by optimal beta-catenin signaling for tumorigenesis. FEBS Lett 580:3665–3670. https://doi.org/10.1016/j.febslet.2006.05.053

Dietlein F, Thelen L, Reinhardt HC (2014) Cancer-specific defects in DNA repair pathways as targets for personalized therapeutic approaches. Trends Genet 30:326–339

Dormand JR, Prince PJ (1980) A family of embedded Runge–Kutta formulae. J Comput Appl Math 6:19–26

Drager A, Hassis N, Supper J, Schroder A, Zell A (2008) SBMLsqueezer: a CellDesigner plug-in to generate kinetic rate equations for biochemical networks. BMC Syst Biol 2:39. https://doi.org/10.1186/1752-0509-2-39

Gavande NS, VanderVere-Carozza PS, Hinshaw HD, Jalal SI, Sears CR, Pawelczak KS, Turchi JJ (2016) DNA repair targeted therapy: the past or future of cancer treatment? Pharmacol Ther 160:65–83. https://doi.org/10.1016/j.pharmthera.2016.02.003

Goldbeter A, Pourquié O (2008) Modeling the segmentation clock as a network of coupled oscillations in the Notch, Wnt and FGF signaling pathways. J Theor Biol 252:574–585

Grady WM, Markowitz SD (2002) Genetic and epigenetic alterations in colon cancer. Ann Rev Genom Hum Genet 3:101–128

Haggar FA, Boushey RP (2009) Colorectal cancer epidemiology: incidence, mortality, survival, and risk factors. Clin Colon Rectal Surg 22:191–197 https://doi.org/10.1055/s-0029-1242458

Hisamuddin IM, Yang VW (2006) Molecular genetics of colorectal cancer: an overview. Curr Colorectal Cancer Rep 2:53–59

Hochman G, Halevi-Tobias K, Kogan Y, Agur Z (2017) Extracellular inhibitors can attenuate tumorigenic Wnt pathway activity in adenomatous polyposis coli mutants: predictions of a validated mathematical model. PloS One 12:e0179888

Huang MY et al (2013) Significant overexpression of DVL1 in Taiwanese colorectal cancer patients with liver metastasis. Int J Mol Sci 14:20492–20507. https://doi.org/10.3390/ijms141020492

Kanehisa M (2002) The KEGG database. Novartis Found Symp 247:91–101 **(discussion 101–103, 119–128, 244–252)**

Katoh M (2007) Networking of WNT, FGF, Notch, BMP, and Hedgehog signaling pathways during carcinogenesis. Stem Cell Rev 3:30–38

Kestler HA, Kuhl M (2008) From individual Wnt pathways towards a Wnt signalling network philosophical transactions of the Royal Society of London Series B. Biol Sci 363:1333–1347. https://doi.org/10.1098/rstb.2007.2251

Kruger R, Heinrich R (2004) Model reduction and analysis of robustness for the Wnt/beta-catenin signal transduction pathway. Genome Inf Int Conf Genome Inf 15:138–148

Lee E, Salic A, Kruger R, Heinrich R, Kirschner MW (2003) The roles of APC and Axin derived from experimental and theoretical analysis of the Wnt pathway. PLoS Biol 1:E10. https://doi.org/10.1371/journal.pbio.0000010

Li SK, Martin A (2016) Mismatch repair and colon cancer: mechanisms and therapies explored. Trends Mol Med 22:274–289

MacLean AL, Harrington HA, Stumpf MP, Byrne HM (2016) Mathematical and statistical techniques for systems medicine: the Wnt signaling pathway as a case study. Systems medicine. Humana Press, New York, 405–439

Markowitz SD, Bertagnolli MM (2009) Molecular origins of cancer: molecular basis of colorectal cancer. N Engl J Med 361:2449–2460. https://doi.org/10.1056/NEJMra0804588

Martin GS (2003) Cell signaling cancer. Cancer Cell 4:167–174

MathWorks T (2012) MATLAB and statistics toolbox release 2012a. The MathWorks, Inc, Natick

Mirams GR, Byrne HM, King JR (2010) A multiple timescale analysis of a mathematical model of the Wnt/β-catenin signalling pathway. J Math Biol 60:131–160

Peltomaki P (2001) Deficient DNA mismatch repair: a common etiologic factor for colon cancer. Hum Mol Genet 10:735–740

Saha S, Roman T, Galante A, Koyuturk M, Ewing RM (2012) Network-based approaches for extending the Wnt signalling pathway and identifying context-specific sub-networks. Int J Comput Biol Drug Des 5:185–205. https://doi.org/10.1504/IJCBDD.2012.049203

Schneikert J, Behrens J (2007) The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut 56:417–425. https://doi.org/10.1136/gut.2006.093310

Shukla A, Sehgal M, Singh TR (2015) Hydroxymethylation and its potential implication in DNA repair system: a review and future perspectives. Gene 564:109–118. https://doi.org/10.1016/j.gene.2015.03.075

Shukla A, Moussa A, Singh TR (2016) DREMECELS: a curated database for base excision and mismatch repair mechanisms associated human malignancies. PloS One 11:e0157031. https://doi.org/10.1371/journal.pone.0157031

Shukla A, Yennamalli RM, Singh TR (2018) Network and structure based inference of functional single nucleotide polymorphisms associated with the TGFβ1 gene and its role in colorectal cancer (CRC). Gene Rep 11:131–142

Sivakumar KC, Dhanesh SB, Shobana S, James J, Mundayoor S (2011) A systems biology approach to model neural stem cell regulation by notch, shh, wnt, and EGF signaling pathways. Omics J Integr Biol 15:729–737

Smaglo BG, Marshall JL (2013) Microsatellite instability in colorectal cancer. Clin Adv Hematol Oncol H&O 11:659–661

Tan CW, Gardiner BS, Hirokawa Y, Layton MJ, Smith DW, Burgess AW (2012) Wnt signalling pathway parameters for mammalian cells. PloS One 7:e31882. https://doi.org/10.1371/journal.pone.0031882

Tariq K, Ghias K (2016) Colorectal cancer carcinogenesis: a review of mechanisms. Cancer Biol Med 13:120–135. https://doi.org/10.28092/j.issn.2095-3941.2015.0103

van Leeuwen IM, Byrne HM, Jensen OE, King JR (2007) Elucidating the interactions between the adhesive and transcriptional functions of β-catenin in normal and cancerous cells. J Theor Biol 247:77–102

Wernicke S, Rasche F (2006) FANMOD: a tool for fast network motif detection. Bioinformatics 22:1152–1153

Young MA, Daly CS, Taylor E, James R, Clarke AR, Reed KR (2018) Subtle deregulation of the Wnt-signaling pathway through loss of Apc2 reduces the fitness of intestinal stem cells. Stem Cells 36:114–122

Zeng ZS, Shu WP, Cohen AM, Guillem JG (2002) Matrix metalloproteinase-7 expression in colorectal cancer liver metastases: evidence for involvement of MMP-7 activation in human cancer metastases. Clin Cancer Res 8:144–148

Zhang W et al (2015) Upregulation of nemo-like kinase is an independent prognostic factor in colorectal cancer. World J Gastroenterol WJG 21:8836