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Cancer Drug Therapy and Stochastic Modelling of “Nano-motors”

Lubna Sherin\textsuperscript{a}, Shebieh Farwa\textsuperscript{b}, Ayesha Sohail\textsuperscript{c,1}, O. A. Beg\textsuperscript{d}, Zhiwu Li\textsuperscript{e,f}

\textsuperscript{a}Department of Chemistry, Comsats Institute of Information Technology, Lahore 54000, Pakistan
\textsuperscript{b}Department of Mathematics, Comsats Institute of Information Technology, Wah Cantt, Pakistan
\textsuperscript{c}Department of Mathematics, Comsats Institute of Information Technology, Lahore 54000, Pakistan
\textsuperscript{d}Fluid Mechanics, Spray Research Group, Mechanical and Petroleum Engineering, School of Computing, Science and Engineering, G77, Newton Building, University of Salford, Manchester, M54WT, UK
\textsuperscript{e}School of Electro-Mechanical Engineering, Xidian University, Xian 710071, China
\textsuperscript{f}Institute of Systems Engineering, Macau University of Science and Technology, Taipa, Macau

Abstract

Controlled inhibition of kinesin motor proteins is highly desired in the field of oncology. Among other interventions, the selective Eg5 competitive and allosteric inhibitors is the most successful targeted chemotherapeutic regime/options, inducing cancer cell apoptosis and tumor regression with improved safety profile. Though promising, this approach is under clinical trials, for the discovery of efficient and least harmful Eg5 inhibitors. The aim of present research is to bridge the computational modelling approach with drug design and therapy of cancer cells. Thus a computational model, interfaced with the clinical data of “Eg5 dynamics” and “inhibitors” via special functions is presented in this article. Comparisons are made for the drug efficacy and the threshold values are predicted through numerical simulations.

Key words

drug efficient model; bipolar spindle; Eg5; cancer; monopolar spindle; Eg5 inhibitors.

\textsuperscript{1}Corresponding author. E-mail address: asohail@ciitlahore.edu.pk
1 Introduction

In recent years mitotic catastrophe activated during mitosis has been employed as promising onco-suppressive therapeutic strategy. In current clinical strategies various antimitotic cancer drugs such as vinca alkaloids, taxanes and epothilone derivatives, targeting microtubules during cell division, have proven to be clinically most successful anticancer agents in bladder, ovarian, breast, lung and head malignancies [1]. Mitosis is a fundamental cell division process that ensures accurate division of sister chromatids to the daughter cells. This vital function, coordinated by mitotic spindle made up of microtubules, has emerged as validated chemotherapeutic target. Microtubules are dynamic polymer of $\alpha/\beta$ tubulin dimer where chromosomes attach and segregate on mitotic spindle during cell division [2, 3]. Antimitotic drugs perturb it by suppressing MT dynamics, thus ensuing an inappropriate chromosome arrangement. Consequently spindle assembly check points are activated that trigger cancer cell death via mitotic arrest [4]. Though MT targeting drugs are currently cancer specific with good clinical outcome but devastating side effects and dose limiting toxicities are the main challenges associated with them as they hit tubulin which is involved in multiple intracellular processes. Moreover, due to innate or acquired resistance patient relapse is a common condition encountered while administrating these drugs [1]. This situation has diverted the research to seek for novel alternative drug targets and develop new generation mitosis specific agents.

Kinesins spindle motor proteins works hand in hand with MT and by virtue of potential therapeutic target in tumors have been studied extensively in this regards. Being central in driving bipolar spindle and subsequent chromosome separation, they provide a chance for the development of specific antimitotic agents that may overcome resistance along with better side effects profile. Kinesins constitute super family of more than 650 known motor proteins that move unidirectional along microtubule to perform exclusive functions and harness energy by ATP hydrolysis in all eukaryotic cells [5]. The key cellular function they perform within intracellular terrain includes microtubule remodeling, vesicular trafficking, mitotic spindle assembly and chromosome segregation in dividing cells [6]. Structure of all kinesins consists of a head, stalk
and tail domain. Head of kinesins contain 340 amino acid motor domain provided with an ATP binding pocket and microtubule binding interface. Being ATPases, kinesins hydrolyze ATP via motor domain to release energy required for the movement along MT. The stalk is used for dimerization and oligomerization while tail is involved in interaction with cargo [7]. Each 8-nm-long tubulin dimer of polymer microtubule serves as one binding site for translocation of kinesin motor domain along microtubule powered by ATP hydrolysis [8].

Studies have shown that human kinesins contribute significantly in cancer development, progression and drug resistance. High expression in various malignancies has directed huge amount of work towards targeting these motor proteins through chemotherapeutic intervention. Abnormal proliferation is the fundamental feature of all tumors. Proliferating neoplastic cells strictly follow well-ordered events of cell cycle comprising of growth S-phase where DNA replication takes place followed by mitosis for cell division. Orderly progression of cell cycle phases is mediated by checkpoints and is stalled under unsatisfied conditions leading to cell death. First identified motor protein was Eg5, a member of kinesin-5 family involved in the establishment of bipolar spindle. Down regulation of this protein results in a defected monopolar phenotype. They are involved in interconnection of chromosomes with spindle, movement of MY dynamics and spindle length maintenance.

1.1 Eg5: An explicit Mitotic Kinesin Motor

Eg5, also known as kinesin spindle protein, KIF11 or Kinesin-5, is the simplest yet key player of the mitotic apparatus. Eg5, a human gene product encoded by KIF11 gene located at 10q24.1, is of particular interest because of its potential as a target for therapeutic intervention [9]. In humans it is expressed in bone marrow, thymus, testis and tonsils while is absent in adult post mitotic central nervous system [10]. It is a homoteterameric plus end directed N-terminal microtubule based motor protein with a catalytic motor/ATPase domain to interact with ATP and microtubules to modulate the dynamics and organization of MT arrays. It can cross link and slide antiparallel MTs to congregate mitotic spindle while it resides along parallel MT at both poles. A defected spindle hampers normal chromosomal segregation leading to mitotic arrest via
check point proteins activation [11]. Metaphase spindle equator comprises of antiparallel overlapped MTs while microtubules with parallel orientation dominate near spindle poles. During bipolar spindle fabrication Eg5 slide apart antiparallel microtubule filaments by pacing towards plus ends of each microtubule. It generates outward pushing force on centrosomes via cross linking antiparallel microtubules to slide them away from each other. Eg5 inhibition arrests cells in mitosis with unseparated centrosomes thus suppressing bipolar spindle production. It is found that Eg5 may also acts as molecular brakes and restricts the movement of overlapping antiparallel filaments. Elongated anaphase spindle has been reported in case of loss of Eg5 activity during cell division [12]. Nevertheless, the magnitude of these braking force has not been directly calculated and scaling of these forces with respect to relative velocity, orientation or overlap is unclear yet. Bipolar architecture of mitotic spindle is critical for proper segregation of chromosomes in daughter cells. The work of Shimamoto et al. provides a conceptual framework towards mitotic apparatus complexity and an insight into self-organization of mitotic spindle. After probing feedback mechanism between Eg5 function and microtubule architecture (velocity and geometry) through experimentations, Shimamoto et al. suggested that spindle assembly
can regulate force generation for self-organization. The forces generated by Eg5 ensembles scale linearly with respect to motor number and length of microtubule overlap. They proposed that Eg5 ensembles can operate as a “force converter”, decoding microtubule regular features such as orientation and overlap length into a distinct force signature. Equator of metaphase spindle is a dynamic region where antiparallel microtubules keep gliding unceasingly at the speed of 23 mm/min (3050 nm/s) while their minus ends are directed towards opposite spindle poles. This dynamic antiparallel setup ensembles of Eg5 generate either pushing or mechanical resistance, magnitude of that is directly proportional to MT sliding velocity, different than unloaded state, and length of overlaid microtubule. A linear integration of force output is attained by plus end movement of Eg5 ensembles in antiparallel geometry while stochastic force output is produced by Eg5 stepping across parallel filaments [13].

Eg5 is assumed as promising therapeutic target as it is over expressed in actively proliferating solid tumors in pancreatic, lung, bladder, ovarian and breast cancers [9, 14]. Elevated levels of Eg5 are considered tumorous as over expression results in blast crisis chronic myeloid leukaemia, activation in mouse B-cell leukaemia, and triggering of genomic instability in transgenic mice [15, 16]. Its oncogenic potential is further verified by the observation that it elicits anti-proliferation of all-trans-retinoic acid in pancreatic cancer cell lines [17]. Experimental data reveal that Eg5 promotes active cancer cell proliferation, colonization, and tumorigenesis in pancreatic malignancy in mice. It was found that Eg5 over expression effected spindle morphology and resulted in multipolar spindles formation. Moreover a significant increase in multinucleate interphase cell population was observed, resulting in accumulation of polyploid cells, a condition strongly linked with genomic instability leading to cancer. Recent studies have shown that kinesin-5 also contributes in evolution of cancer towards metastases via spindle length scaling [18]. Studies of many cancer cell lines along with in vivo human xenograft models have shown that Eg5 inhibition ceases bipolar mitotic spindle morphogenesis and ends up in loss-of-function phenotype as a monopolar spindle monoester with chromosomes distribution in rosette like configuration. This mitotic spindle disaster activates checkpoint proteins induces mitotic arrest and subsequent apoptosis of cancer cell [19, 20].
Upregulation of Eg5, a potent biomarker in various malignancies, needs to be targeted for timely and efficacious treatment. In contrast to traditional antimitotic drug targeting MT in actively proliferating as well as normal cells, severe side effects are not expected from Eg5 inhibitors for being target specific for mitotically active cells. Small molecules inhibitors targeting Eg5 signify a new generation target specific anti-cancer agents that are currently undergoing phase I and phase II clinical trials [21]. Mitotic arrest has been induced by these inhibitors by obstructing Eg5-dependent MT motility resulting in aberrant monoastral form. The first reported potent Eg5 inhibitor was monastrol that caused mitotic arrest without interfering with MT dynamics. Afterwards a large number of allosteric inhibitors has been reported belonging to different chemical classes such as quinazoline, imidazoles, thiadiazoles, carbolines, dihydropyrazoles, isoquinolines and benzimidazoles.

1.2 In silico Biology for representing stochastic intracellular Changes

Stochastic description of intracellular changes is well-demonstrated in computational systems biology. However, several models are usually generalization of the actual phenomena and the corresponding parameters may be imprecise. In such cases, the numerical tools can help to investigate the analysis results and their level of sensitivity corresponding to the parametric perturbations. In this paper, a stochastic simulation algorithm is adopted to analyse the variation in dynamics of the kinesin motor proteins in three cases (normal, cancerous and drug treated cells). We aim to provide the reader, a simple yet dynamic model to demonstrate the self-organisation of stochastic time stepping of the kinesin motor proteins under aforementioned conditions.

2 Problem Statement

The study of motor proteins and microtubules is a rich field of research with a long list of open problems. Different approaches are available in the recent literature to model the stochastic transport, mitotic spindle dynamics in cancerous cells and the self-organization of sub cellu-
lar structures, the receptor trafficking, protein-DNA interactions, nuclear transport, membrane diffusion and virus trafficking. This motivates us to study and compare change in dynamics of Eg5 motor proteins in normal and highly proliferating cancerous cells in addition to change exhibited by diseased cells in the presence of obstacles and transient traps i.e. inhibitors. In normal cells, metaphase spindle is highly dynamic entity. Being made up of microtubules and associated kinesin motor proteins, it experiences huge fluctuations and directed fluxes in both physical as well as chemical processes. However, average number, position and functions of all spindle constituents remain steady overtime by virtue of its ability to correct transient changes. In cancerous cells firmness of this steady-state is disturbed and it experiences huge physical and chemical perturbations with evident incapability to recover or to correct transient fluctuations in morphology and position. Consequently an aggressive, unchecked mitotic division takes place leading to high proliferation rate of tumor cells.

This entire process is really rapid and the laboratory experiments alone are not enough to capture the rapid alterations caused by randomness. The literature provides an evidence that the computational biology (in-silico research) has always served successfully to model the inter and intracellular dynamics of malignant cells [22]. This motivates us to use a computational model with some advanced conditions, in order to demonstrate the “Eg5 motor proteins based malignant cell’s intra-dynamics”. The research methodology encompass the stochastic behaviour of Eg5 proteins and its response to both the tumour disorders (proliferations) and the drug therapy. In this article, after careful analysis of the clinical trials, we have selected a special type of inhibitor “Ispinesib (I_{sp})” which is believed to control the over expression of the motor protein Eg5 [23, 18, 21] in tumour cells. After the selection, we have extended the stochastic model available in the literature [24, 25, 26] inline to the given biological problem. We have considered the Hill function formalism to deomstrate the addition of drug in the model as a reflection of its role in the laboratory generated experiments [23]. We have presented some results based on the mathematical model and the parametric values to demonstrate the efficiency of the computational tool to make important predictions. This will help to improve the ongoing clinical trials on the control of Eg5 via inhibitors [27, 28] in cancerous cells.
3 Kolmogorov’s Backward Equation

The kinesin motor proteins are involved in the mitotic cell division. The modeling and simulation of kinesin dynamics and their mobility along the microtubules is really challenging in a normal (case 1), tumour (case 2) and treated cell (case 3). There are several factors involved, such as, a variation in the frequency of the release of the proteins hence change in force and motion produced via catalysis in three different cases, there can be various time scales in operation, and the entire process is inherently stochastic. The Markov process (which is also famous as “Chemical Master Equation (CME)” framework) is appropriate to conduct such an analysis.

For the clear understanding of the molecular motor proteins dynamics, in this article, a discrete approach is adopted. We have considered the stochastic model to describe the dynamics, the probability of the molecule in a certain \( t^{th} \) state at a time \( t \) can help to demonstrate the dynamics, provided that the backward kolmogorov equation governs its transient dynamics.

Let \( \mathcal{X}_t \) represent an Itô diffusion in \( \mathcal{R}^n \), having generator \( \mathcal{A} \). Choosing a function \( h \in C_0^2(\mathcal{R}^n) \) and taking \( \tau = t \) in Dynkin’s formula, we have

\[
\nu(t, x) = E^x[h(\mathcal{X}_t)]
\]  

(1)

It is obvious that \( \nu(t, x) \) is differentiable with respect to \( t \) and

\[
\frac{\partial \nu}{\partial t} = E^x[\mathcal{A}h(\mathcal{X}_t)]
\]  

(2)

In above equation, \( E^x[\mathcal{A}h(\mathcal{X}_t)] \) can be expressed in terms of \( \nu \).

**Theorem 3.1.** Let \( h \in C_0^2(\mathcal{R}^n) \) and \( \nu(t, x) \) be as defined above.

1. Then for every value of \( t, \nu(t,.) \in \mathcal{D}_\mathcal{A} \) and

\[
\frac{\partial \nu}{\partial t} = \mathcal{A}\nu, \ t > 0, \ x \in \mathcal{R}^n
\]  

(3)

\[
\nu(0, x) = h(x), \ x \in \mathcal{R}^n
\]  

(4)
2. Besides above, if \( \mu(t,x) \in C^{1,2}(\mathcal{R} \times \mathcal{R}^n) \) is a bounded function, for which both the aforementioned eq.2 and 3 hold, then \( \mu(t,x) = \nu(t,x) \) as given in eq.(1).

4 Setting and Definitions

Taking into account an \( n \) – dimensional stochastic differential equation

\[
d\mathcal{X} = \rho(\mathcal{X})dt + \zeta(\mathcal{X})dW(t), \quad \mathcal{X}(0) = \mathcal{X}_0
\]

(5)

Let \( \mathcal{E} \) be the space that may be specified as either \( \mathcal{E} = \mathcal{R}^n \) or the torus \( \mathcal{E} = \mathcal{T}^n \). In above equation, \( \mathcal{X}_0 \in \mathcal{E} \) is the initial condition (for simplicity, we assume deterministic), and \( W(t) \) is a standard \( d \) – dimensional Weiner process. Both the maps \( \rho : \mathcal{E} \rightarrow \mathcal{E} \) and \( \zeta : \mathcal{E} \rightarrow \mathcal{E}^d \) are considered smooth. Sometimes, we preferably use the vector notation for the matrix \( \zeta(x) \), as \( \zeta(x) = (\zeta^1(x), \zeta^2(x), ..., \zeta^d(x)) \), where each of \( \zeta^i \in \mathcal{E} \)

Consider a discrete numerical approximation of eq.(5) given as follows

\[
\mathcal{X}_{i+1} = \Phi(\mathcal{X}_i, \kappa, v_i),
\]

(6)

where \( \mathcal{X}_i \in \mathcal{E}, \forall i \geq 0 \) and \( \Phi(., \kappa, v_i) : \mathcal{E} \rightarrow \mathcal{E} \) is the discrete numerical flow, \( \kappa \) stands for the time-step size and \( v_i \) represents a random vector.

Numerical Integration

Associated with the Stochastic Differential Equation (SDE), as stated by eq.(5), its generator \( \mathcal{A} \) is a differential operator given by,

\[
\mathcal{A} = \rho \cdot \nabla + \frac{1}{2} \zeta \zeta^T : \nabla^2
\]

(7)

The above equation represents the scalar product of two matrices \( M = \rho \cdot \nabla + \frac{1}{2} \zeta \zeta^T \) and \( N = \nabla^2 \), where \( M : N = \text{trace}(M^T N) \) and \( \nabla^2 h \) is the Hessian of the function \( h \in C_0^2(\mathcal{R}^n) \) and thus the
function $\nu(t, x) = E^x[h(X_t)|X_0 = x]$ is the solution of the Kolmogorov’s backward equation

$$\frac{\partial \nu}{\partial t} = A\nu, \quad t > 0, \ x \in \mathbb{R}^n, \ \text{with} \ \nu(0, x) = h(x), \ x \in \mathbb{R}^n.$$ 

Taking a Taylor expansion along with the Kolmogorov’s backward equation (as stated above), we obtain the following series for $\nu(x, t)$ that involves the generator $A$ of the SDE (given by eq.(5))

$$\nu(x, \kappa) - h(x) = \sum_{m=1}^{l} \frac{\kappa^m}{m!} h(x) + \kappa^l e_l(\rho, \zeta, h)(x), \quad (8)$$

where $e_l(\rho, \zeta, h)$ is the remainder and under appropriate conditions regarding the smoothness of $\rho, \zeta$ and $h$, it may follow polynomial growths ($\mathcal{E} = \mathbb{R}^n$) or may be bounded ($\mathcal{E} = \mathcal{T}^n$). For the numerical method, we consider the function

$$\hat{\nu}(x, \kappa) = E^x[h(X_1)|X_0 = x] \quad (9)$$

Assuming that the above expression can be expanded as

$$\hat{\nu}(x, \kappa) = h(x) + \kappa \mathcal{L}_0(\rho, \zeta) h(x) + \kappa^2 \mathcal{L}_1(\rho, \zeta) h(x) + ... \quad (10)$$

with $\mathcal{L}_i(\rho, \zeta)$ denote the linear differential operators depending upon the choice of the integrator, having coefficients relying on $\rho, \zeta$ and their respective derivatives. We further assume that

$$\mathcal{L}_i(\rho + \eta \hat{\rho}, \zeta + \eta \hat{\zeta}) = \mathcal{L}_i(\rho, \zeta) + \eta \mathcal{L}_i(\rho, \hat{\rho}, \zeta, \hat{\zeta}) + \mathcal{O}(\eta^2) \quad (11)$$

where $\mathcal{L}_i(\rho, \hat{\rho}, \zeta, \hat{\zeta}) + \mathcal{O}(\eta^2)$ is also a differential operator. It is clear from $|E^x[h(X_1)] - E^x[h(X_{t_1})]| = |\hat{\nu}(x, \kappa) - \nu(x, \kappa)|$, that for every method of local weak order $p \geq 1$, the consistency condition must hold, i.e. $\mathcal{L}_o = A$. In addition to this, the following equation is satisfied

$$|E^x[h(X_1)] - E^x[h(X_{t_1})]| = \kappa^{p+1} (\mathcal{L}_p - \frac{\mathcal{L}_p}{(p+1)!}) h(x) + \mathcal{O}(\kappa^{p+2}) \quad (12)$$

It is worth-mentioning that a global weak order result can also be given in terms of the above stated differential operators. This leads us to the following result:
**Theorem 4.1.** In eq.(5), let $\rho$ and $\zeta \in C^\infty$ with bounded derivatives of any order and take a numerical integrator (eq.(6)) on $[0, T]$, having an expansion as stated by eq.(10) with bounded moments $E^x[X_j]_{\gamma}$ for sufficiently large $\gamma \in \mathbb{N}$. If assumed that the numerical integrator has weak local order $p$ with a constant $C = C(x)$, following polynomial growth then $\forall h \in C_0^2(\mathbb{R}^n)$, the expansion for the global error is given by

$$E^x[h(X(T))] - E^x[h(X_0)] = \kappa^p \int_0^T E^x[\Phi_e(X(\theta), \theta)]d\theta + \mathcal{O}(\kappa^{p+1}), \quad (13)$$

where $S\kappa = T$ and $\Phi_e(t, x)$ satisfies

$$\Phi_e(t, x) = (\mathcal{L}_p - \frac{A^{p+1}}{(p + 1)!})\nu(t, x), \quad (14)$$

where $\nu(t, x)$ is the solution to eq.(3).

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5 **In-vivo & In-vitro Studies and Theoretical Model**

In this section, we have discussed some experimental studies and have interfaced these with our theoretical model.

5.1 **Data Based Stochastic Effects of EG5 Inhibitors**

5.1.1 **Tumour Cells and Eg5 Data**

Oncogenic role of Eg5 has been established by a large body of experiments and it is identified as a potent prognostic biomarker in various malignancies. Meiping Lu et al. investigated the correlation between up-regulated expressions of Eg5 to clinicopathological characteristics in laryngeal squamous cell carcinoma (LSCC) patients. For that purpose immunohistochemistry (IHC) analysis in 137LSCC cases along with one-step qPCR test with 20 fresh-frozen LSCC samples was done. Significant higher Eg5 proteins level in comparison to corresponding non-cancerous tissues were correlated with lymph node metastasis and TNM stage, independent factors to envisage...
critical prognosis for LSCC patients [29]. Similarly correlation between clinicopathological characteristics and Eg5 expression in non-muscle invasive urothelial carcinoma was investigated by analyzing large number of immunohistochemistry specimens including grade: G1, 32 cases; G2, 92 cases; and G3, 39 cases. Stage: pTa, 49 cases and pT1, 114 cases. 163 non-muscle invasive cases were analyzed via survival analysis to find out the prognostic significance of Eg5 immunoreactivity. A strong imperative connection between Eg5 over expression and tumor grade (P = 0.006) and tumor stage (P = 0.057) was discovered. It was concluded that Eg5 overexpression signifies a self-regulating prognostic factor in envisaging initial intravesical recurrence in non-muscle invasive bladder cancer patients [14]. Dingqi sun et al evaluated the prognostic significance of Eg5 up regulation in renal cell carcinoma (RCC) patients via immunohistochemistry and correlated clinicopathological parameters using the univariate and multivariate analysis of 164 patients and 164 tissue specimens, regularly followed from 5 to 80 months. They suggested that Eg5 may serve as a prognostic factor for renal cancer prediction, evolution and appropriate treatments. Experimental data indicated significant relation between tumor nuclear grade (P = 0.019), stage (P = 0.007) and size (P = 0.033) with Eg5 expression for recurrence free RCC patient survival [30]. Eg5 expression has also been associated with occurrence and rapid metastasizing ability of pancreatic cancer. In pancreas cancer cell lines, high expression of Eg5 promotes tumorigenesis by boosting cell proliferation in an ATPase activity-dependent manner. It stimulates multipolar spindle formation and consequent multi-nucleation leading to polyploid cells production. It was discovered that Eg5 high expression encourages anchorage independent cell growth and tumor metastases in mice model [9].

5.1.2 Eg5 Data after Inhibition

Due to close association of Eg5 with tumorigenesis, metastasis and tumor drug resistance, Eg5/KSP has emerged as a promising target for anticancer therapeutic agents and various KSP inhibitors are under clinical trials as monotherapy or adjuvant therapy candidate. Eg5 inhibitors cause cell cycle arrest during mitosis by targeting KSP via contrasting mechanisms. ATP-uncompetitive inhibitors bind near loop L5 to stabilize the bound nucleotide and trap the
motor in a weak binding state [Ref 34]. Some potent inhibitors of this category exhibiting clinical efficacy include monastrol, filanesib, litronesib, K858, S-trityl-L-cysteine (STLC) and ispinesib ($I_{sp}$) [23, 31, 32, 33]. Whereas thiazole FCPT act as ATP competitive inhibitor and bind directly to the nucleotide binding active site [34]. Some biaryl inhibitors such as PVZB1194 and GSK-133 act as allosteric competitive inhibitors of ATP binding and bind near the α4α6 interface [33, 35]. BRD9876 is a similar type of ATP non-competitive inhibitor that preferentially bind to microtubule bound Eg5 [36]. These inhibitors may act as a chemical probe to understand and modulate the Eg5 activity within a cell. Detailed analysis depicted different distinct effects of aforementioned inhibitors on MT stability and spindle integrity. L5 inhibitors obliterate Eg5s microtubule stabilizing ability causing metaphase spindle down fall in contrast to rigor inhibitor BRD9876 that stabilizes microtubule and hence metaphase spindle by suppressing MT depolymerization.

5.2 Computational Model Interfaced with Real Data

In the recent literature [37], the motor proteins and their associated stochastic dynamics are studied extensively due to their crucial role, specifically, in mitotic cell division. These motor proteins are mostly believed to obey periodic motion. Such motion usually takes place along the potential, which actually describes the distinct biochemical states of the motor. There are several types of motor proteins involved in mitosis process in human cell. Among these, EG5 motor proteins play a dominant role in the cancer invasion. It has been verified through laboratory generated experiments, (as reported by Wakui et al. [23]) that EG5 is over expressed in malignant cells. We have focused on the dynamics of this special type of motor proteins using a theoretical model (that is explained in detail in sections 3 & 4), where $\nu(x, t)$ represents the probability (or more precisely the probability density) for EG5 to be found at location $x$ at time $t$. The conditions to solve the Kolmogorov’s Backward equation, were considered to be periodic. Since at the micro level, the probability density function can be written as a function of mean square displacement, therefore as the mean square displacement of EG5 proteins changed, we obtained a variation in the conditions interfaced in the numerical solver. This can be elaborated
with the aid of the probability density function

\[
\nu = \frac{e^{\frac{-\Xi}{k_BT}}}{\int_{x} \int_{p} e^{\frac{-\Xi}{k_BT}} dx dp}
\]  

(15)

where \( p \) is the momentum of the motor protein (depending on the rate of change of the displacement), \( \Xi \) is the sum of kinetic and potential energies (as a function of displacement), \( k_B \) is the Boltzmann constant and \( T \) is the absolute temperature. In this article, the parametric values were adapted from the data presented by Chen et al [38].

Another important feature of this study is that, the drug therapy is synchronized in the model through the Hill’s function formulation [39]. The EG5 molecules were considered as the enzymatic receptors and the drug molecules were considered as the inhibiting ligands. The simple manipulation of the chemical equations leads to an important formula, which is used extensively in the literature [40], but for the very first time has been synchronized with the sinusoidal drift (\( \rho \) in Eq. 5) of the Kolmogorov’s Backward equation. The Hill function for our model is derived using the following formalism:

**Hill Functions**

The reaction, in which \( g \) ligand molecules \( I_{sp} \) bind the receptor \( Eg5 \), is given as follows

\[
Eg5 + gI_{sp} \rightleftharpoons Eg5gI_{sp}
\]  

(16)

In case of chemical equilibrium, the following relation is satisfied

\[
[Eg5] + [I_{sp}]^g = R_{Eg5}[Eg5gI_{sp}]
\]  

(17)

In the above equation \( K \) represents the reaction dissociation constant and \([Eg5]\) and \([I_{sp}]\) stand for the concentration of the chemical species \( Eg5 \) and \( I_{sp} \) respectively. If we consider a constant
number of receptors,

\[ [Eg5] + [Eg5gIsp] = [Eg5Tot] \]  \hspace{1cm} (18)

Eq. (16) and (17) then lead to the following fractions:

\[ \mathcal{H}^{(1)}([I_{sp}]) = \frac{[Eg5gIsp]}{[Eg5Tot]} = \frac{[I_{sp}]^g}{K^g + [I_{sp}]^g} \text{ and } \mathcal{H}^{(2)}([I_{sp}]) = \frac{[Eg5]}{[Eg5Tot]} = \frac{K^g}{K^g + [I_{sp}]^g} \]  \hspace{1cm} (19)

where both \( \mathcal{H}^{(1)} \) and \( \mathcal{H}^{(2)} \) are known as Hill functions, representing the fraction of occupied and free molecules \( Eg5 \) respectively, with \( K^g = R_{Eg5} \). It should be noted that \( \mathcal{H}^{(1)} \) is a monotonic increasing function of \( [I_{sp}] \), that satisfies the following properties

1. \( \mathcal{H}^{(1)}(0) = 0 \)
2. \( \mathcal{H}^{(1)}(K) = 1/2 \)
3. \( \lim_{[I_{sp}] \to \infty} \mathcal{H}^{(1)}([I_{sp}]) = 1 \)

However \( \mathcal{H}^{(2)} \) is a monotonic decreasing function of \( [I_{sp}] \) satisfying the following conditions

1. \( \mathcal{H}^{(2)}(0) = 1 \)
2. \( \mathcal{H}^{(2)}(K) = 1/2 \)
3. \( \lim_{[I_{sp}] \to \infty} \mathcal{H}^{(2)}([I_{sp}]) = 0 \)

Further \( \mathcal{H}^{(1)}([I_{sp}]) + \mathcal{H}^{(2)}([I_{sp}]) = 1 \). Now let’s define \( x = [I_{sp}]/K \), then the normalized Hill function is stated as

\[ h^{(1)}(x) = \mathcal{H}^{(1)}(Kx) = \frac{x^g}{1 + x^g} \text{ and } h^{(2)}(x) = \mathcal{H}^{(2)}(Kx) = \frac{1}{1 + x^g} \]  \hspace{1cm} (20)

This non-dimensional entity \( h^{(1)}(x) \) when incorporated with the sinusoidal drift \( (\rho \text{ in Eq. 5}) \) of the Kolmogorov’s Backward equation works as a useful tool to trace the effect of the drug
Figure 2: Drug therapy theoretical strategy and the impact on drift.
inhibitors on the Eg5 enzymes. This impact is demonstrated with the aid of graphical interpretation in figure 2.

We have solved the Kolmogorov backward equation to understand the three cases, kinesin motor proteins dynamics in normal cell, tumour cell and treated cell. Each case was differentiated from the other case, based on the initial dynamics (interfaced in the numerical computations of system 3 via variation in 4 and 15 in the associated parametric values, such as the drift. The finite element algorithm was applied and the mass and stiffness matrices were obtained. The matrix exponential is computed using a scaling and squaring algorithm with a Pade approximation.

5.3 Results and Discussion

In this section, we have presented some results based on the experimental findings and numerical simulations.

Figures 3, 4 depicts the probability density function for the motor proteins in a normal cell and in a cell with malignancy. We can see that the shape changes from regular soliton like structure to frequent humps.

From figures 5 and 6 it is obvious that for lower values of $g$ (that controls the drug concentration in our model), there is a decline in amplitude of the pathway and for increased values (7) it reduces asymptotically and the straight line after $x = 12$ in figure 7 shows apoptosis, i.e. no motion of the motor protein.

6 Conclusions

Mathematical modeling of the stochastic intracellular perturbations in the expression and function of Eg5 in malignancy and on inhibition may prove to be fruitful in understanding the current challenges of cellular scale studies. In this model, an elevated expression of Eg5 generates excess mechanical forces for the movement of anti-parallel microtubules and perturbs the balance of forces normally required for bipolar spindle formation, thereby inducing multipolar spindle
Figure 3: The probability density function for the motor proteins in a normal cell.

Figure 4: The probability density function for the motor proteins in a tumour cell when no drug was injected.
Figure 5: States of motor protein inside a cancerous cell for lower dose of drug.
Figure 6: States of motor protein inside a cancerous cell for increased dose of drug.
Figure 7: States of motor protein of a treated cell, for enhanced drug dose, the straight line refers to no movement (i.e. necrosis).
assembly and impairing chromosome segregation. The cells may undergo mitotic slippage due to the inability to correct errors and satisfy the spindle assembly checkpoint, resulting in cytokinesis arrest, polyploidy, and abnormal proliferation. These events in turn lead to genomic instability and ultimately tumourgenesis. This model display the aggressive stochastic behavior of kinesin-5 in cancerous cells and tamed behavior upon inhibition. Further studies are warranted to understand the precise mechanisms that underlie the role of Eg5 in the development of cancer that will be a crucial step towards its therapy.

study and compare change in stochastic dynamics of Eg5 motor proteins in normal and highly proliferating cancerous cells in addition to change exhibited by diseased cells in the presence of obstacles and transient traps i.e. inhibitors.

In this article we have emphasized on the fact that the stochastic modeling of the dynamical behavior of motor proteins can help to predict the outcomes of cancer drug therapy. The inhibition of Eg5 motor protein, a key mitotic kinesin motor protein involved in aggressive mitotic activity of cancerous cells is targeted in this study to elaborate this statement. The mathematical model is physically motivated by the existing models as well as the laboratory outcomes and thus presents the dynamics to a better accuracy. We aim to extend this study by taking into account the mechanochemical properties of the motor proteins and the self alignment of the motor proteins.

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