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CYCLIN-DEPENDENT KINASES AS DRUG TARGETS FOR CELL GROWTH AND PROLIFERATION DISORDERS. A ROLE FOR SYSTEMS BIOLOGY APPROACH IN DRUG DEVELOPMENT. PART II – CDKs AS DRUG TARGETS IN HYPERTROPHIC CELL GROWTH. MODELLING OF DRUGS TARGETING CDKs

Michael A. Idowu
University of Abertay Dundee, School of Contemporary Sciences, Scottish Informatics Mathematics Biology and Statistics (SIMBIOS) Centre, Scotland, UK
Correspondence to: Michael A. Idowu
E-mail: m.idowu@abertay.ac.uk

ABSTRACT

Cyclin-dependent kinases (CDKs) are key regulators of cell growth and proliferation. Impaired regulation of their activity leads to various diseases such as cancer and heart hypertrophy. Consequently, a number of CDKs are considered as targets for drug discovery. We review the development of inhibitors of CDK2 as anti-cancer drugs in the first part of the paper and in the second part, respectively, the development of inhibitors of CDK9 as potential therapeutics for heart hypertrophy. We argue that the above diseases are systems biology, or network diseases. In order to fully understand the complexity of the cell growth and proliferation disorders, in addition to experimental sciences, a systems biology approach, involving mathematical and computational modelling ought to be employed.

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Targeting CDK9 for cardiac hypertrophy drug development

Unlike CDK2, CDK9 is not directly involved in cell cycle regulation but promotes RNA synthesis and thus controls cell growth, differentiation and viral pathogenesis. CDK9 inhibition contributes to the anticancer activity of most CDK inhibitors currently under clinical investigation (25, 33, 46). In addition, the activity of CDK9 is up-regulated in myocardial hypertrophy. The latter constitutes a common clinical finding as well as a prominent risk factor in congestive heart failure. Thus, CDK9 inhibitors may find therapeutic application in cardiology.

CDK has been shown to be successfully readily inhibited by Roscovitine and flavopiridol *in vitro* as well as *in vivo* (1, 4, 28, 29, 40). Novel selective CDK9 inhibitors have been reported recently (48). In addition, in order to test the effect of transcriptional inhibition on cardiac hypertrophy, a highly reproducible animal model has been developed and characterized (18, 37, 38, 39). A simple technique such as abdominal aorta binding is applied so as to cause left ventricular hypertrophy (LVH) in male Wistar rats and subsequently echocardiography is used as a non-invasive method for evaluation of cardiac function. The main advantage of this model is that it is technically uncomplicated and undemanding as it may be performed in the most commonly used laboratory animals (Wistar rats). Since the technique does not call for thoracotomy it has the additional advantage that the model animals do not need a long recovery period.

Role of modelling in developing drugs targeting CDKs

The process of developing drugs for diseases of the cell cycle and predicting the effect *in vitro* and *in vivo* requires an analysis and comprehensive study of their targets including concentration levels, activities, interactions and involvement in biochemical networks. Nowadays it is becoming clear that a disease of the cell cycle is a systems biology disease, or a network disease. This means that it is the regulatory network that is dysfunctional so that the cell escapes normal growth control by its multicellular environment. To fully understand the complexity of the problem, mathematical and computational modelling can be utilized in ways which will add value to the data obtained from the experimental sciences such as biochemistry and molecular and cell biology. The results may be surprising. For example, for the last two decades, the common condition of insulin resistance has been identified to have an inflammatory pathogenesis. Only in 2005 it has been reported that at least some of the cases of severe diabetes type 2 in animals and humans were related to deficiency of the master transcription HMGA1 (17). During the transition between S and G2 in the cell cycle, in non-cancerous cells CDK2 phosphorylates HMGA1, decreasing its DNA-binding activity and facilitating its habitual shuttling from the nucleus to the mitochondria, and then back by M phase (7, 14, 34). Since very low expression of 'normal' HMGA is associated with insulin resistance and perhaps with premalignant states (9, 17, 33), it is possible that inhibition of CDK2 could be used as a therapeutic option in other diseases and conditions apart from cancer. It has already been demonstrated that CDK inhibitors such as Roscovitine are capable of reducing the pro-inflammatory response in chronic inflammation via down-

regulation of NF-kappaB (15, 23). It is possible that the anti-inflammatory properties of Roscovitine and CDK inhibitors as a whole may find a significant clinical application. There has already been some experimental proof that the inhibition of CDK5 by Roscovitine enhances the induction of insulin secretion and exerts a protective effect against glucotoxicity in pancreatic beta cells (25, 41, 50). Therefore, inflammatory diseases such as diabetes type 2 turn out to be network diseases as well, which opens new vistas of therapeutic opportunities as well as of potential downsides.

Establishing an appropriate cell cycle model is a purpose-driven process, which in particular is focused on the key questions of the investigation. The demands for novel analytic computational and modelling strategies that keep up with the ever-increasing supply of minable time series data are rising all the time. In this respect, robust dynamic modelling techniques are useful for the simulating and analysing biomarker data.

The dynamics of a complex network of regulatory proteins that govern cell cycle progression, involved with biochemical switches that initiate cell-cycle events can be modelled by nonlinear ordinary differential equations describing the essential network responses to internal signals among regulatory proteins. The progression through the cell cycle system can be predicted using modelling approaches for capturing the relevant molecular processes and suited for easing the process of estimating kinetic parameters. To avoid additional complexity, some researchers often consider experimenting and modelling cell cycle regulation based on single cell studies.

Mathematical modelling of biological phenomena, involving biochemical networks (44, 45) analysis of clinical or biomolecular data (8, 24) spanning genetics (6, 16, 36) and personalised medicine (31, 32) may be used to describe and represent complex systems. Usually this involves formulating appropriate functions that describe the behaviour of interacting constituents of the system.

Identification of components of interest with their symbolic names with (directional) arrows showing which components modulate the flows of signal and materials into, between, and out of components (44) may involve similar methods such as method of gene network reconstruction from microarray data identification of unknown targets of drug candidates from gene products (5, 8). Bansal et al. (2, 3) inferred gene regulatory networks from time course gene expression profiles using an algorithm they developed called time-series network identification (TSNI). Also, Gardner et al. used network identification by multiple regression (NIR) method to infer genetic networks and identify compound mode of action via expression profiling (10, 19). Wang et al. developed and presented a linear programming framework for inferring gene regulatory networks by integrating multiple perturbation expression datasets (20, 49). Likewise, modelling and network inference procedures have been used in all different types of networks: metabolomics, protein-interaction, signal-transduction networks, and genetic regulatory networks. Examples include the use of graphical networks, nonlinear differential equations and bifurcation theory for the description of the regulation of cell cycle protein interactions (11, 12, 13). Novak and Tyson (18, 27, 30) offered a generic wiring diagram of the CDK network by explaining the various signal and response mechanisms that can occur as part of a biological control system, including sigmoid response, positive and negative feedback, and oscillations with a description of the mathematical structures in each case.

In the search for appropriate mathematical representations of biological processes, e.g. using rational functions of the Michaelis-Menten rate law as ordinary differential equation (ODE) fundamental descriptions, it is essential to bear in mind the importance of keeping to absolute minimum variables and parameters that traditionally cannot be directly measured (44). Although many biological processes, including those involved in the control and coordination of cell-cycle can be described with such formalism, its inability to easily articulate the dynamic model structure and the problematic nature of estimating its kinetic parameters have made it unattractive. For example, the Michaelis-Menten formalism is not consistent enough in demonstrating some fractal kinetics observed at the system's molecular level. Rather than constraining model structure to occasional restrictions imposed by the Michaelis-Menten formalism, others have adopted a more flexible formalism called Power-Law, which is based on a general Biochemical Systems Theory (BST) (42, 44, 46). Idowu et al. (22) proposed a novel data-driven approach using a combination of analytical methods and Power-law formalism based on the requirement that these alternative mathematical forms, based on time series data, are amenable to analytical and numerical evaluation (44).

Experimental time series data of the system are obtained and assumed to be snapshots of time-evolutionary dynamics of the underlying activities of the biological processes. The modelling challenge is to accurately capture and represent the majority of the system dynamics in a data-consistent manner without adopting a model that is mathematically too complex and without making a priori assumptions about the underlying network. Power-law models are good examples of mathematically and logistically convenient models (44). Another much simpler alternative that is backed up by strong theoretical support and justification is the data-consistent Jacobian model proposed by Idowu and Bown (21) which offers system identification and parameter estimation solutions through two new algorithms, namely the transposive and repressive regression techniques.

Viewing the modelling strategies of the cell cycle control system as an investigative science, it is often necessary to critically evaluate common intellectual contributions that are influential or detrimental to their outcome. Simple manual processes involving predetermination of network topology and formulation of model structure with preconfigured kinetic parameters are very common. Though they often yield additional benefits to the modeller's experience in the process, if they are introduced too early at the stage of model design, the spectrum of all potential possibilities and outcomes may be hugely affected with negative consequences. The notion that

the relevant informative aspect of modelling should be driven by a secure process rather than subverting towards existing biological knowledge should be appreciated (11, 12, 43).

A lot has changed in recent times compared to lack of cohesion of computational, mathematical, and experimental approach to modelling observed many years ago: quantitative data are rapidly becoming more and more available; the rate at which experimental data is shared is growing; there is increasing awareness about the importance of interdisciplinary collaborative work and an increasing number of qualified researchers from computational, physics, mathematics and engineering areas now engage in systems biology projects. Vast amount of funds are invested into academic research and development, both at local as well as at international level.

However, the requirement to develop new and sophisticated mathematical techniques for modelling the molecular concentration dynamics of key proteins (e.g. CDKs and cyclins) of the cell cycle control system continues to be on high demand due to the increasing amount of quantitative data. Accommodating the rising trend in data supply requires a shift of mind set towards dealing and coping with the huge costs of carrying out biological experiments. Not only that, circumventing the systemic problems commonly associated with system identification and model parameter estimation processes may require some level of thinking 'outside the box'.

A combinatorial approach that merges creative thinking, science and research into traditional and unconventional modelling methods may help in identifying and validating protein targets. Understanding diseases of the cell cycle requires new unconventional methods targeted towards creating generic modelling techniques, an optimised modelling strategy for data consumption and utilization and a development of effective and robust network reconstruction procedures. Expectation to see more data-mining-powered modelling tactics being established across multidisciplinary areas continues to rise.

In the immediate future, more demand will be placed on network inference algorithms to uncover the underlying network of interactions among interrelated components. As growth and development of research into understanding network complexity, model assessment, system identification, parameter estimation, stability and sensitivity analyses and robustness measurement reach maturity, the performances of these network inference methods would improve. Application of generic data-driven and data-consistent dynamic could potentially become common and useful in harnessing information hidden in data. However, since the cell cycle control system involves a complex and highly nonlinear dynamics, the type of modelling framework used should be flexible to a degree of accommodating different types of data sets.

Validating CDKs as targets for disease of the cell cycle involves evaluating and understanding some of the key roles that CDKs play in cell cycle regulation. One way of demonstrating this might be to run in simulations of data-consistent and individualised models of non-treated and treated cells with comparison tests. The results of these analyses should seek to identify changes in response behaviour in CDKs which may result in toxicity after drug treatment. Alterations in levels of CDKs may vary from time to time, both before and after treatment and from cell to cell.

In summary, involvement of modelling may assist in creating robust (self-organizing and self-calibrating in terms of data set variation and unit of measurement) models in order to demonstrate the extent to which personalised model kinetic parameters may vary between normal and cancer data set, before and after treatment. The resultant changes in regulatory network topology after treatment should enrich the current understanding of a system's response and sensitivity to drugs. Individualised and data-consistent models of time series measurements obtained from the same tissue sample may then be viewed as dissociated building blocks of a much bigger compartmentalised model of all the associative data sets. However, it is unclear how to go about reorganising and integrating all the parts into one coherent and reliable working unit. Having said that, the complex behavioural patterns and complex oscillations and chaos often exhibited in cell-cycle control systems complicates the difficulty of grasping the full understanding of key roles that protein targets play in cell cycle regulation. Although some theoretical methodologies capable of tackling these may be available in the literature, a lot still depends on the ingenuity of the modellers. Each modelling approach adopted by the modeller should be considered on the basis of the set of vital questions that are being addressed.

Conclusions

Investigating the essential mechanisms by which the cell cycle control system operates is an important step in drug discovery, which may help reveal vital information to support and facilitate new drug target identification. Based on a good understanding of the key protein functions and their roles in cell cycle control, new and effective drugs may be developed.

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