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Drug target identification in sphingolipid metabolism by computational systems biology tools: Metabolic control analysis and metabolic pathway analysis

F. Betül Kavun Özbayraktar *, Kutlu Ö. Ülgen

Department of Chemical Engineering, Bogazici University, 34342 Bebek, Istanbul, Turkey

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ABSTRACT

Sphingolipids regulate cellular processes that are critically important in cell's fate and function in cancer development and progression. This fact underlies the basics of the novel cancer therapy approach. The pharmacological manipulation of the sphingolipid metabolism in cancer therapeutics necessitates the detailed understanding of the pathway. Two computational systems biology tools are used to identify potential drug target enzymes among sphingolipid pathway that can be further utilized in drug design studies for cancer therapy. The enzymes in sphingolipid pathway were ranked according to their roles in controlling the metabolic network by metabolic control analysis. The physiologically connected reactions, i.e. biologically significant and functional modules of network, were identified by metabolic pathway analysis. The final set of candidate drug target enzymes are selected such that their manipulation leads to ceramide accumulation and long chain base phosphates depletion. The mathematical tools' efficiency for drug target identification performed in this study is validated by clinically available drugs.

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1. Introduction

Sphingolipids comprise a class of complex lipids which are abundantly found in cell membrane. These membrane lipids do not only function as structural components of the cell membrane but they possess important roles in signal transduction (as second messengers) and in regulatory pathways such as cell cycle arrest, apoptosis, senescence and differentiation. The investigation of the processes that are regulated by bioactive sphingolipid signaling molecules demonstrates that sphingolipids are fundamental to cancer pathogenesis and therapeutics [1]. Ceramide, an essential building block of sphingolipids, has been suggested to be a reasonable key metabolite in cancer therapy inducing antiproliferative and apoptotic responses. Many anti-cancer drugs are reported to lead to increased endogenous ceramide levels. Ceramide's influence on cell growth is dynamically balanced by sphingosine-1-phosphate and this balance is termed as "sphingolipid rheostat". In cancer cells, this dynamic balance is missing [1–8].

A novel cancer therapy approach resides on the adjustment of the sphingolipid metabolism to accumulate ceramide and to decrease sphingosine-1-phosphate based on the fact of ceramide being a "tumor-suppressor lipid" and sphingosine-1-phosphate being a "tumor-promoting lipid". The drugs using this fact are expected to resemble the antiproliferative and apoptotic responses in the cell by altering the sphingolipid levels. The advantages of such changes are not only in cancer therapy but also, enhancement of drug action and chemoprevention [1,6–8].

There are several newly developed synthetic inhibitors of specific enzymes involved in sphingolipid metabolism [9]. The target enzymes of human sphingolipid pathway are serine palmitoyl transferase, ceramide synthase, ceramidase, sphingosine kinase, glucosylceramide synthase, 1-O-acylceramide synthase. A number of drugs like N-4-hydroxyphenyl retinamide (4-HPR), Valspodar (PSC 833), daunorubicin, ceramide analog (B13), safingol, butyldeoxynojirimycin and phenoxodiol targeting the above listed enzymes are in pre-clinical or clinical development [7,9–12]. Changing ceramide levels in favor of cancer therapy seems to be a promising strategy if the developed drugs targeting the newly identified sphingolipid enzymes are selective toward malignant cells but not healthy cells. With some ceramidase inhibitor trials this selectivity seems to be possible due to the hypersensitivity of malignant cells to ceramide perturbation [7]. Another evidence for the significance of accumulation of ceramide in cancer cells is that cancer cells escape from apoptosis by degrading, catabolizing or converting ceramide [9]. Experimentalists observed elevated levels of acid ceramidase expression in human prostate cancer cell lines [13]. The manipulation of these cellular processes gives the chance of developing new anti-cancer therapeutics and indeed, forms the basics of the novel cancer therapy method [1].

The most difficult step in drug discovery is the decision of the drug target enzymes. Since clinical aspects of sphingolipids are still

* Corresponding author. Fax: +90 212 287 24 60.
E-mail address: betulkavun@gmail.com (F.B.K. Özbayraktar).

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lacking information, the identification of drug targets by employing computational tools is a significant requirement. Metabolic control analysis is a powerful mathematical tool to solve this puzzle of drug target identification via considering the role of individual components within a metabolic network [14]. As mentioned previously, sphingolipids regulate processes that are vital in terms of cell's fate. These processes are all very important for cancer initiation, progression and treatment [6]. The identification of critical enzymes within sphingolipid metabolism facilitates the link between sphingolipids and cancer.

In the present study, the key points of sphingolipid metabolism in terms of ceramide and sphingosine-1-phosphate are investigated using two different mathematical frameworks, metabolic control analysis and metabolic pathway analysis. Metabolic control analysis is based on dynamic data whereas metabolic pathway analysis uses the stoichiometry of the system. These two systems biology tools reveal the important metabolic steps of the sphingolipid pathway and using this information novel drug target enzymes can be recognized and potential drug target enzymes in human pathway can be proposed. Although the *Saccharomyces cerevisiae* and human sphingolipid pathways are similar to each other, one should admit that *S. cerevisiae* sphingolipid pathway consists of only few complex sphingolipids when compared with that of mammalian cells/human. Thus, this study can be considered as an attempt to bridge the basic sphingolipid research to potential medical applications in cancer therapy.

2. Computational methods

In order to gain a better understanding of sphingolipid metabolism, *S. cerevisiae* is taken as the model system. The availability of the detailed and complete enzymatic and genetic data of *S. cerevisiae* makes the organism eligible for computational works that can give reliable first guesses for human studies. The conservation of sphingolipid metabolic and regulatory pathways across species has been used in the identification of the corresponding mammalian orthologues [15–18]. In the present study, the protein sequence homologies of the enzymes that are responsible for the sphingolipid pathway activity were cross-checked against human (*Homo sapiens*) proteome via NCBI (National Center for Biotechnology Information) protein blast utility. Almost all the enzymes involved in the sphingolipid reactions of the model system have a homolog in human proteome with a reasonable and exceptable E-value, which shows the similarity of the two protein sequences. Also the functional domains of the yeast and human sphingolipid homolog proteins were compared and almost complete overlap is observed. The *de novo* sphingolipid metabolism of yeast exactly matches to that of human with the exception of the double bonded ceramide in human which is missed in yeast cells. The complex sphingolipids of yeast and human differ but the potential drug targets proposed in the current study correspond to mainly *de novo* sphingolipid synthesis.

2.1. Sphingolipid metabolic pathway

This model system consists of three parts. The main part is the sphingolipid metabolism and the two auxiliary pathways are the fatty acid metabolism and phospholipid metabolism. They integrate sphingolipid metabolism to other cellular processes. The fatty acid metabolism reactions used in the model system are steps that correspond to the synthesis and elongation of fatty acids. The very long chain fatty acids (C_{26}-CoA) are employed as precursors in the synthesis of dihydroceramide (DHCer) and phytoceramide (PHCer). Phospholipid metabolism is also another important branch point at the initial step of sphingolipid, i.e. at palmitoyl-coenzyme A (Pal-CoA) level. Synthesis of phosphatidyl inositol (PI) is included in the model system due to the fact that; PI is a precursor metabolite for the synthesis of complex sphingolipid, inositol phosphorylceramide (IPC) from both dihydroceramide and phytoceramide.

The sphingolipid metabolic pathway (Table 1) for the dynamic model is taken from Alvarez-Vasquez et al. [19] and explained below. The first reaction of the *de novo* sphingolipid synthesis is the condensation of serine and Pal-CoA via the enzyme serine palmitoyl transferase, r_{11}, and takes place in the endoplasmic reticulum. This reaction produces the precursor metabolite 3-ketodihydrosphingosine (KDHS) which is required for the synthesis of the first long chain base, dihydrosphingosine (DHS). KDHS is rapidly reduced to the long chain base, r_{13}, catalyzed by 3-ketodihydrosphingosine reductase in the endoplasmic reticulum. The condensation reaction can be named as the initializing step of *de novo* sphingolipid synthesis in *S. cerevisiae* [2,16,17].

After the formation of the sphingoid long chain bases in *S. cerevisiae*, there are two alternative paths that the long chain bases can follow: acylation or phosphorylation. Dihydrosphingosine (DHS) and phytosphingosine (PHS) are acylated to DHCer, r_{23}, and PHCer, r_{24}, respectively, by ceramide synthase and the reactions take place in endoplasmic reticulum [16,19,20]. The enzyme responsible for the phosphorylation of the long chain bases to long chain base phosphates is sphingoid base kinase which catalyzes the synthesis of dihydrosphingosine-1-phosphate (DHS-P) and phytosphingosine-1-phosphate (PHS-P), r_{15} and r_{20}, respectively. The reverse reactions, dephosphorylations, r_{14} and $r_{19,}$ are also possible by the enzyme sphingoid-1-phosphate phosphatase in endoplasmic reticulum of *S. cerevisiae*. The phosphorylation and dephosphorylation steps are needed for the synthesis of sphingolipids. The phosphorylated long chain bases can be decomposed by the enzyme sphingosine-phosphate lyase to cytidine diphosphate-ethanolamine (CDP-E), r_{17} and r_{18}. At this point sphingolipid pathway is again linked to the phospholipid pathway [16,19].

DHCer and PHCer can be converted back to long chain bases of DHS and PHS, r_{22} and r_{25}, by dihydroceramide and phytoceramide alkaline ceramidases in the endoplasmic reticulum [16,19]. Hydroxylation of DHS and DHCer to PHS, r_{21}, and PHCer, r_{26}, respectively, are catalyzed by sphingosine hydroxylase encoded by *SUR2* in endoplasmic reticulum [20]. This enzyme is responsible for the adjustment of balance between the two *S. cerevisiae* sphingoid long chain bases [16,19]. This step does not take place in mammalian cells, where dihydroceramide is desaturated to form ceramide but not phytoceramide [1].

In *S. cerevisiae* three types of complex sphingolipids containing *myo*-inositol are synthesized: inositol phosphorylceramide (IPC), r_{29} and r_{30}, mannosylinositol phosphorylceramide (MIPC), r_{39}, and mannosyldiinositol phosphorylceramide ($M(IP)_2C$), r_{49}. As the only alteration of ceramide in *S. cerevisiae* is by the addition of inositol phosphate (IP), these three complex sphingolipids are the end products of *S. cerevisiae* sphingolipid metabolism. This modification by IP occurs only in *S. cerevisiae* but not in mammalian cells; therefore IPC, MIPC, $M(IP)_2C$ may not be found in mammalian cells. In *S. cerevisiae*, complex sphingolipid synthesis reactions are reported to occur in the Golgi apparatus [2,16].

There are 49 irreversible reactions in *S. cerevisiae* sphingolipid metabolism. The enzymes corresponding to each reaction are listed in Table 1. Thirty-one metabolites with their abbreviations are given in Table 2. The rate equations are taken from Alvarez-Vasquez et al. [19] who used Biochemical Systems Theory (BST) and Generalized Mass Action (GMA) representation. The rate expressions for each reaction represent the effect of the substrate concentration,

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