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ORIGINAL

Genome-scale reconstruction and systems analysis of brain microglial cells

Reconstrucción a escala del genoma y análisis de sistemas de células microgliales cerebrales

Haleh Iranmanesh

1. Department of Biotechnology, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

Corresponding author
Haleh Iranmanesh
E-mail: Halehiranmanesh@gmail.com

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Abstract

Introduction and objectives: Microglia are neuroglial cells found throughout the brain and spinal cord. The present study aimed to perform a metabolic comparison between normal and activated microglial cells.

Materials and methods: In the present study, the GEO database was used to access the transcript data. After selecting the appropriate data for the type of cell culture and cell treatment and how to obtain the relevant tissue, and based on the format type of these data, the corresponding package was selected from the Bioconductor and the data were normalized. P-Values were determined to evaluate the significance of statistical studies and the GIMME algorithm and GLPK linear programming solver was used in MATLAB software.

Results: Two types of samples were used in this study: normal and activated microglia. Comparison of the studied models showed that there were some metabolic differences between the two types of microglia, including the number of presented genes, the number of metabolites, and the number of metabolic reactions. The results of this study showed a decrease in the activity of both aconitase and arginase enzymes in activated microglia compared to normal microglia. On the other hand, gene expression associated with succinyl coagulase and lactate dehydrogenase as well as the activity of nitric oxide synthase increased in activated microglia, but the synthesis of fatty acids decreased in the deactivated state.

Conclusion: Reconstruction of the microglia metabolic network based on genomic, biochemical, and physiological data provides an overall outlook of cellular metabolism.

Keywords: Genome-scale of metabolic networks, brain, microglia, systemic medicine.

Resumen

Introducción y objetivos: Las microglías son células neurogliales que se encuentran en todo el cerebro y la médula espinal. El presente estudio tiene como objetivo realizar una comparación metabólica entre células microgliales normales y activadas.

Materiales y métodos: En el presente estudio se utilizó la base de datos GEO para acceder a los datos de transcripción. Tras seleccionar los datos apropiados para el tipo de cultivo celular y el tratamiento celular y la forma de obtener el tejido pertinente, y en función del tipo de formato de estos datos, se seleccionó el paquete correspondiente del Bioconductor y se normalizaron los datos. Se determinaron los valores P para evaluar la importancia de los estudios estadísticos y se utilizó el algoritmo GIMME y el solucionador de programación lineal GLPK en el software MATLAB.

Resultados: En este estudio se utilizaron dos tipos de muestras: microglía normal y activada. La comparación de los modelos estudiados mostró que había algunas diferencias metabólicas entre los dos tipos de microglía, incluyendo el número de genes presentados, el número de metabolitos y el número de reacciones metabólicas. Los resultados de este estudio mostraron una disminución de la actividad de las enzimas aconitasa y arginasa en la microglía activada en comparación con la microglía normal. Por otra parte, la expresión génica asociada a la succinil coagulasa y a la lactato deshidrogenasa, así como la actividad de la óxido nítrico sintasa, aumentaron en la microglía activada, pero la síntesis de ácidos grasos disminuyó en el estado desactivado.

Conclusión: La reconstrucción de la red metabólica de la microglía basada en datos genómicos, bioquímicos y fisiológicos proporciona una visión global del metabolismo celular.

Palabras clave: Red metabólica a escala genómica, cerebro, microglía, medicina sistémica.

Introduction

Microglia are neuroglial cells found throughout the brain and spinal cord[1]. These cells form 20% of the total population of glial cells, which account for 10 to 15% of the brain cells[2,3]. The microglia are originated from hematopoietic stem cells in the spinal cord, some of which differentiate into monocytes and then into microglia after migrating to the brain and residing in the tissue[4]. Microglia are considered the first and most important line of defense in the active immune system in the CNS because they are a type of tissue-resident macrophages[5]. They act as the main inflammatory cells in the brain and respond to pathogens and brain damage by transforming into activated form and through phagocytosis[5]. The response of these cells is the secretion of substances such as cytokines and chemokines, prostaglandins, nitric oxide, and reactive oxygen species[5].

In recent years, the development of high-throughput laboratory methods has led to the formation of large genome-scale databases for a variety of organisms and tissues[6]. Microarray technology enables researchers to study the gene expression patterns of cells and tissues. Therefore, using these new techniques makes it possible to identify cell components. Regularization of biological systems requires comprehensive models (systemic modeling) of cellular processes[7]. Hence, a rational method to achieve a biological understanding of complex datasets is via mathematical modeling, quantitative simulation, and analysis of their results. In recent years, notable efforts have been made toward developing genomic-scale metabolic models for many organisms. These models are based on new techniques and several analytical tools are developed for computational and quantitative analysis of modeled organisms[8]. In 2007, two general models of human metabolism were published that are widely used in systematic studies[9].

To date, various genome-scale metabolic models have been reconstructed for a variety of human tissues, including the genome-scale reconstruction of the astrocyte metabolic network. Among the most famous reconstructions for human tissue are various efforts for different types of cancers. Moreover, several other models have been developed for different tissues of the human body based on Recon 2. The analysis of these models using biological system methods can provide tremendous information on bioenergetic interactions and causal mechanisms underlying the aggressive behavior of these cells when invading central nervous system cells. Biological systems allow for integrating data from all levels of biology, including genomics, proteomics, and metabolomics, and analyzing them in the context of systems theory and control, thus can provide an in-depth understanding of the underlying processes of cell behavior, including microglia. The present study aims to develop the first genome-scale model of microglia cells and examine it according to the existing knowledge on the physiology of these cells. Therefore, the study was designed and conducted to reconstruct and systematically analyze the genome-scale metabolic network of the brain microglia cells.

Materials and methods

Data collection from databases

In the present study, the GEO database was used as the data source to access transcript information. This database contains transcript data from the microarray technique for a variety of tissues and microorganisms. After selecting the appropriate data for the type of cell culture and cell treatment and how to obtain the relevant tissue, and based on the format type of the data, the corresponding package was selected from the Bioconductor and the data were normalized. P-Values were determined to evaluate the significance of statistical studies and the GIMME algorithm was then applied. This algorithm eliminates the reactions with the least flux in the relevant tissue and maintains the minimum necessary reactions in the model based on the phenotype.

Model building based on the constraints

The process of model building and analyzing can be divided into four consecutive steps: **1.** reconstruction of the metabolic network, **2.** formation of the stoichiometric matrix, **3.** define and assign proper constraints on molecular components, and **4.** network analysis. The process of network reconstruction can be divided into three simultaneous sections: 1. data collection to define the target network, 2. preparing a list of all metabolic reactions within the cell, and 3. determination of gene-protein-reaction (GPR) relationships.

Solving the linear programming problem

Linear programming is a mathematical method to find the minimum or maximum value of a linear function on a convex polygon[10]. There are various algorithms for linear programming. In the present study, the GLPK algorithm was used as a free solver in MATLAB software. Moreover, to reduce the range of samples and resulting errors, sampling predictions were applied in the present study[11]. This process was performed for metabolic fluxes and the sampling results, including median, mean, and standard deviation of metabolic fluxes, were used to match previous library data.

Results

The main purpose of this study was to perform a metabolic comparison between normal (m0) and activated (m1) microglia cells. To this end, appropriate samples of microglia gene expression data were first obtained from the GEO database. These data were obtained from

microarray operations on microglia cells derived from the brain of an adult human. The microarray chip data was [HuGene-2_0-st] [HuGene-2_0-st] Affymetrix Human Gene 2.0 ST Array [transcript (gene) version]. Next, the data were normalized to achieve a list of genes that were considered to be present or absent according to the specified cut-off (P-value = 0.05).

As previously mentioned, two types of samples were used for modeling in the present study, including normal and activated microglia. Comparison of these models indicates the metabolic difference between these two types of microglia, which is visible at the genomic level. Among the apparent differences in model, structure is the number of presented genes, the number of metabolites, and the number of metabolic reactions. In general, these two types of microglia have certain metabolic differences, as presented in **table I**.

Table I: Structural differences between the metabolic models of normal and activated microglia.

genes	metabolites	reactions	Cell type
1857	2733	4954	M0
1874	2747	4988	M1

Since a decrease in aconitase activity is associated with increased cis-aconitate and decreased isocitrate, the best approach to measure these changes in metabolic models is to examine the flux sampling data of reactions in the two models and compare the mean values of the fluxes (**Table II**).

Table II: Calculation of the mean flux difference of the aconitase reaction.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
26.2444	3658.1	'ACONT'	cit[c] <=> icit[c]	'Citric Acid Cycle'	'aconitase'

The difference in mean reaction fluxes indicates a decrease or increase in enzyme activity. As articulated in previous literature, there is a decrease in the activity or down-regulation of the aconitase enzyme in M1 compared to M0. Based on previous studies[12,13], only activated microglia shows an angiogenic-like phenotype and the expression of the SUCLG2 gene, indicating the generation of mitochondrial succinyl-CoA. Therefore, we expect an increase in gene expression related to succinyl-CoA ligase in activated microglia (**Table III**).

Table III: Calculation of the mean flux difference of the succinyl-CoA ligase reaction.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
-30.6021	8801.1	'SUCOAS1m'	'coa[m]+gtp[m]+succ[m] <=> pi[m]+gdp[m]+succoa[m] '	'Citric Acid Cycle'	'Succinate--CoA ligase (GDP-forming)'

According to previous studies[14], when microglia are activated, we expect an increase in the lactate dehydrogenase-related gene expression (**Table IV**).

Table IV: Mean flux difference of the lactate dehydrogenase.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
-208.216	160287.1	'LDH_L'	nad[c] + lac_L[c] <=> ' h[c] + pyr[c] + nadh[c] '	Glycolysis/' 'gluconeogenesis	'lactate dehydrogenase'

A review of the literature showed that the overall profile of fatty acids decreases during inflammation and activation of microglia[15]. In a deactivated state, the synthesis of fatty acids is lower due to the presence of rate-limiting agents of malonyl-CoA, which is the substrate of the fatty acid synthase enzyme (**Table V**).

Table V: Mean flux difference of fatty acid synthase.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
-4.11215707	FASN	'FAS100COA'	'3 h[c] + 2 nadph[c] + malcoa[c] + occoa[c] -> h2o[c] + 2 nadp[c] + co2[c] + coa[c] + dcacoa[c] '	'Fatty acid synthesis'	'fatty acyl-CoA synthase (n-C10:0CoA)'

According to the literature review[16,17], it is expected to occur an increase in nitric oxide synthase activity in activated microglia and, conversely, an increase in arginase activity in deactivated microglia. In the present study, as expected, there was a decrease in the arginase-related reaction flux in M1, indicating a decrease in the activity of the enzyme arginase (**Table VI**).

Table VI: Mean flux difference of arginase activity.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
0.28380	'ARGN'	'R00551'	h2o[c] + arg_L[c] -> orn[c] ' ' + urea[c]	'Arginase'	'Urea cycle'

Since the mean flux difference is negative, we concluded that the reaction flux of nitric oxide synthase in M1 is higher than M0, so the difference is less than zero (**Table VII**).

Table VII: Mean flux difference of nitric oxide synthase reaction.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
-11.4605906	'NOS2'	'R00557'	o2[c] + nadph[c] + ' nwharg[c] -> h2o[c] + h[c] + nadp[c] + citr_L[c] ' + no[c]	Arginine ' and Proline 'Metabolism	Nitric Oxide ' Synthase (NO 'forming)

The main characteristic of microglia M1 is the production of reactive oxygen species (ROS) (17). It is expected to see a decrease in pyruvate dehydrogenase activity in microglia M1 (**Table VIII**).

Table VII: Mean flux difference of pyruvate dehydrogenase reaction.

Mean flux difference M0-M1	Gene ID	Reaction name	Reaction	Pathway	Enzyme
-594.4252	'PDHm'	'R01699'	'coa[m] + ' nad[m] + pyr[m] -> co2[m] + nadh[m] + ' accoa[m]	'Glycolysis /gluconeogenesis'	'Pyrovate dehydrogenase'

Discussion

Genome-scale analysis of microglial cells can reveal their metabolic capacity, the biosystem mechanisms of invasion as well as the mechanism of transformation into neurons, and lead to strategies to prevent or treat the neuro-destructive effects of these cells. In the present study, two types of samples were used for modeling in the present study, including normal and activated microglia. Comparison of these models indicates the metabolic difference between these two types of microglia, which is visible at the genomic level. However, the most important metabolic differences are presented below, which are consistent with previous studies, indicating the accuracy of the developed models. As mentioned previously, various statistical tools were used to compare the two models of this study. Our comparative method was based on calculating and comparing the mean flux differences of each metabolic reaction between the two models. According to previous studies, the common symptoms of neurodegenerative diseases are microglia activation, oxidative stress, impaired mitochondrial energy metabolism, and accumulation of intracellular iron. Microglia activation is a common process in neurodegenerative diseases. The essential role of nitric oxide in the incidence and continuation of neurodegenerative disorders and activation of microglia has been discussed in several studies[18-20]. However, it should be noted that although the presence of nitric oxide is essential to maintain the iron cycle, its excessive increase is problematic. Long-term production of nitric oxide leads to suppression of cytochrome C oxidase and reduced activity of complexes I and II. As a result of these two processes, ATP levels decreased, while ATPase activity remained unchanged. Nitric oxide and peroxynitrite inhibit several mitochondrial enzymes, including respiratory chain complexes and a Krebs cycle enzyme called aconitase[12,21,22]. According to these studies, the first difference in microglia M1 compared to M0 is expected to be a decrease in aconitase activity. Recent studies on the metabolic status of the central nervous system during the development of neurodegenerative diseases have shown that the release of lactate dehydrogenase from existing cells is associated with increased inflammation and angiogenesis[14].

Autophagic activity is one of the main characteristics of microglia because microglia are a type of tissue-resident macrophage. This process disrupts in the case of neurodegenerative diseases and aging. Research has been conducted on metabolic processes involved in autophagy, disruption of which is manifested as activated microglia phenotype. When exposed to microbes and foreign substances, microglia undergo an oxidative burst, producing reactive oxygen-based compounds that kill the microbes[16]. The association between oxidative burst and reorientation of arginine metabolism has been investigated in previous studies[17]. According to these studies, an increase in the content of nitric oxide causes metabolic disorders, and to reduce the subsequent damage, more arginine is secreted into the phagocytic cell to form a blood clot in the area. After entering the cell, arginine follows one of the following two pathways. If nitric oxide is present in the cell environment, arginine is affected by the enzyme nitric oxide synthase, producing citrulline. If arginine enters a cell with a normal condition, it is affected by the arginase enzyme and produces urea and ornithine. Accordingly, it is expected to observe an increase in nitric oxide synthase activity in activated microglia and, conversely, an increase in arginase activity in deactivated microglia.

Another key feature of microglia M1 is the production of reactive oxygen species (ROS)[17]. These species enable the phagocytic cell to kill pathogens. As the glutathione reduction process begins, some NADPH is produced that prevents further ROS damage to the intercellular area[23]. Meanwhile, NO species are produced with the occurrence of arginine oxidation by the NOS enzyme and using electrons derived from NADPH[17]. At high concentrations, NO competes with oxygen in cytochrome C oxidase, disrupting mitochondrial respiration. As mitochondrial respiration decreases, ROS species are increased into superoxide anion and converted to hydrogen peroxide by superoxide dismutase, which is finally transported to the cytoplasm. With the advance of this process, NO reacts with the superoxide anion to produce peroxynitrite, which inhibits the enzyme pyruvate dehydrogenase. This enzyme produces acetyl CoA from pyruvate, which later enters the Krebs cycle. Therefore, it is expected to occur a decrease in pyruvate dehydrogenase activity in M1 microglia.

Conclusion

Reconstruction of the microglia metabolic network based on genomic, biochemical, and physiological data provides an overall outlook of cellular metabolism. In this

study, a genome-scale metabolic model of microglia was developed that can be used in future studies, and gap-filling operation can also be applied to this model in the suitable cultural medium for microglia. To increase the accuracy of such models, it is recommended to use valid transcript data, i.e., using diverse samples in the microarray technique and donors with high age diversity. Moreover, other algorithms can also be used to systematically study and analyze other neurodegenerative disorders for future research.

Interests conflict

The authors declare no conflict of interest.

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