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Extracting Cancer specific reactions using Cancer Metabolic Networks

Abhijit Paul[1] Shyam lal Bagaria[1] Rajat Anand[1] Samrat Chatterjee[1*]

[1]Computational and Mathematical Biology Lab, Drug Discovery Research Centre, Translational Health Science and Technology Institute, NCR Biotech science cluster, 3rd milestone, Faridabad-Gurgaon expressway, Faridabad-121001, India.

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Abstract. *There are many drugs that target cancer cells and kill them, but with side e ects. The reason is still the lack of understanding of how a cancer develops from a normal tissue. Detailed understanding could help in making better drugs that will have fewer side e ects. On the other hand, it is being increasingly recognized that a major hallmark of cancer is altered metabolism. We, thus, in this work, attempted to find the crucial metabolic reactions which are perturbed in cancer using available metabolic networks of cancer. We performed our analysis for cancer metabolic networks on different tissue type to find cancer specific reactions which are independent of tissue of origin and compared their flux values with their normal tissue counterparts. The cancer specific reactions found in our study could be as a starting point for experimental biologists to capture possible mechanism for cancer.*

1 Introduction

Cancer is a global burden affecting millions of population [1–3]. Cancer affects many tissues such as leukemias, melanomas, ovary, renal, breast, prostate, colon, lung and central nervous system(CNS). Many drugs are made to target cancer cells and kill them. However, such drugs usually have high side effects [4, 5]. Part of the reason behind this is the lack of detailed understanding about **the** mechanism behind the development of cancer in a tissue. Such understanding can help in designing better therapies against cancer [6, 7]. It is being increasingly acknowledged that one of the hallmarks of cancer is altered metabolism [8]. We thus aimed to find the critical reactions that are perturbed in cancer which could possibly be tested in experiments for their possible causal role in developing cancer.

Metabolic networks of cancers with different tissues of origin are available in literature [9]. These networks contain the reactions regulating in the corresponding cancer tissue based on the expression levels of their corresponding catalyzing proteins taken from high-throughput data. We asked in this study whether we could analyze these networks to find reactions potentially causing cancer**.**

We thus, in this work took the already made metabolic networks of cancer to find the reactions with high rates across all cancer tissue types. We then used the metabolic networks of normal tissue types [10] to find reactions specific

[*]Corresponding author: Samrat Chatterjee, Email: samrat.chatterjee@thsti.res.in

to normal tissues and used this to find reactions specific to cancer. We finally discuss the implications of our findings in terms of mechanistic understanding of cancer.

2 Material and Methods

2.1 Flux Balance Analysis

For flux balance analysis, MATLAB was used and gpSampler function from the COBRA package [11] was used.

3 Result

3.1 Metabolic networks of cancer tissues and normal tissues

We used the study by Yizhak et al. [9] to obtain the metabolic networks of cancer of different tissue types. We also obtained the metabolic networks of normal tissue types from Thiele et al. [10]. In total, we obtained 60 cancer metabolic networks across 9 tissue types and 8 normal metabolic **networks** for 8 tissue types.

3.2 Flux balance analysis on metabolic networks

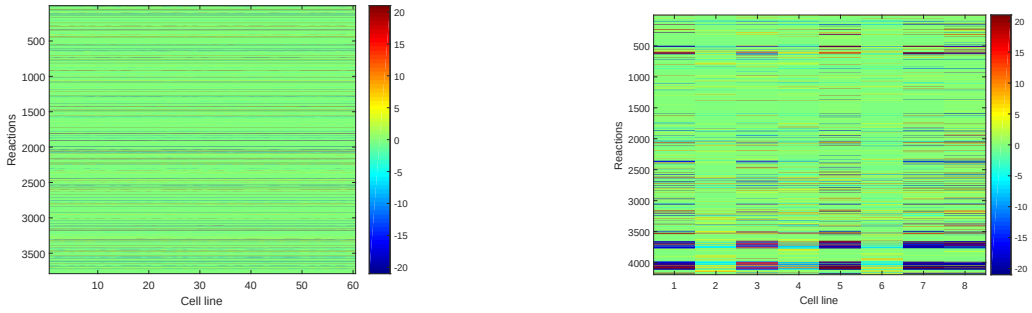
We used an established methodology of flux balance analysis [12] to find the rates of each of the reactions present in the metabolic networks of normal and cancer cells. Briefly it solves the equation :

$$S.v = 0 \tag{3.1}$$

where, S is the stoichiometric matrix of size $m \times n$, m is the number of metabolites and n is the number of the reactions present in the metabolic network. Each element of the matrix contains the stoichiometric coefficient of the metabolites participating in a reaction. V is a $n \times 1$ matrix consisting of the flux value of the reactions and each component V_i satisfies $V_{i_{min}} \leqslant V_i \leqslant V_{i_{max}}$ and the bounds in the rate of each reaction $(V_{i_{min}}, V_{i_{max}})$ given in the models. This is done for the case of normal cells.

In cancer cells the requirement of proteins, lipids, nucleotides and **energies** were found to be very high for enhanced growth and proliferation [13, 14]. For the case of cancer cells, an additional objective function is added where the growth rate of cancer cells is maximized as done in [9].

Since the solution to Eq. 1 is not unique, a distribution of flux values of each reaction in each network is obtained and mean values are used. This way, we obtain the flux values of each reaction across all metabolic networks. A heat map of the flux values in cancer networks is shown in Fig. 1A and for normal networks in Fig. 1B .



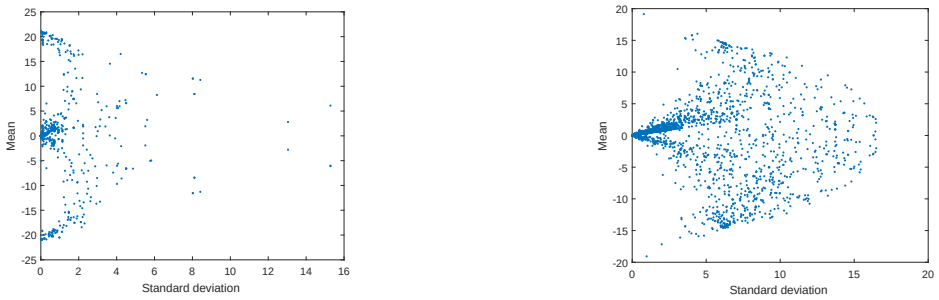
(a) Cancer cell lines

(b) Normal cell lines

Fig. 1: Heat map of the flux values of reactions in (A) cancer networks and in (B) normal tissue networks.

3.3 Finding reactions specific to cancer

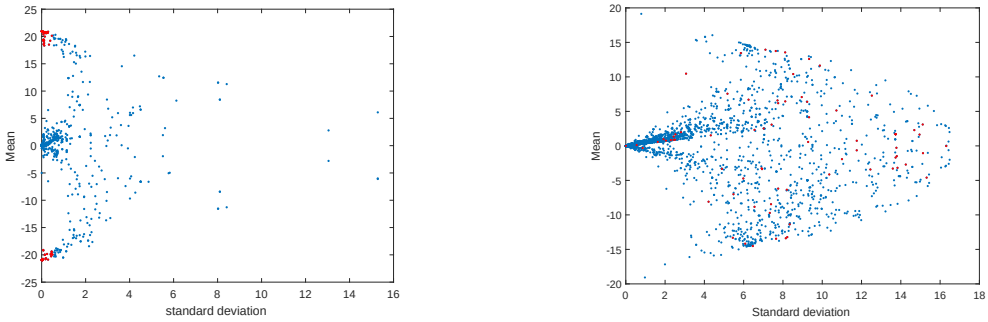
To find reactions specific to cancer, we reasoned that reaction with high flux across all cancer cell lines and same reaction shows zero flux across all normal cell lines could be important. The reactions with high flux across all cancer cell lines and varying flux across normal tissues could also be important. To systematically find such reactions, we calculated the mean flux value and standard deviation of flux values of each reaction across all cancer metabolic networks and plotted them in Fig. 2A . We repeated the same exercise for networks of normal cell lines as shown in Fig. 2B .



(a) Cancer cell lines (b) Normal cell lines

Fig. 2: Mean vs Standard deviation plot of each reaction across (A) cancer cell lines and (B) normal cell lines.

From, here the reactions with high absolute mean in cancer cells (absolute values greater than 15) and standard deviation less than 0.5 were considered as **the** reaction with high absolute flux across cancer cells. There are 89 reactions. These are highlighted in mean vs standard deviation plot of cancer cells Fig. 2A and shown in Fig. 3A . Out of these 89 cancer reactions, 74 reactions were present in normal cell lines. To know these reactions behavior in **the** normal cells, we highlighted these reactions in **the** mean vs standard deviation plot of normal cells Fig. 2B and **showed** in Fig. 3B .



(a) Cancer cell lines (b) Normal cell lines

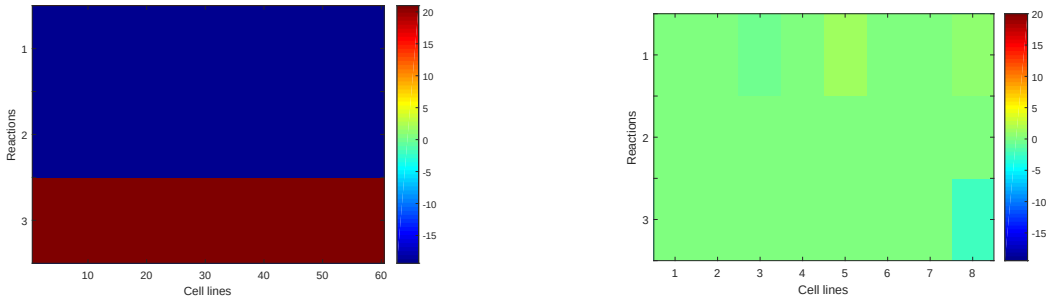
Fig. 3: Mean vs Standard deviation plot of the (A) 89 number of reactions across cancer cell lines and of the (B) 74 number of reactions in normal cell lines which represent by the red dots in the picture.

Now from these set of reactions using a threshold value cut off of 1 on standard deviation of flux values in normal cell **lines,** we got 3 reactions whose standard deviation of flux values lie below the cutoff value. Interestingly, we show that these 3 reactions have very low flux values in the normal cell lines. So, we obtained 3 reactions that shows high flux values across all cancer cell lines and very low values in all normal cell lines. The names of the reactions are given in Table 1.

Table 1: Reactions having high flux in all cancer cell lines but negligible in all normal cell lines.

acetyl-CoA C-acetyltransferase
fatty-acid–CoA ligase (or synthetase)
phosphate transport in/out via two Na+ symporter

A heat map of the flux distribution of the above mentioned three reactions in cancer cell lines is shown in Fig. 4A and for normal cell lines in Fig. 4B.



(a) Cancer cell lines (b) Normal cell lines

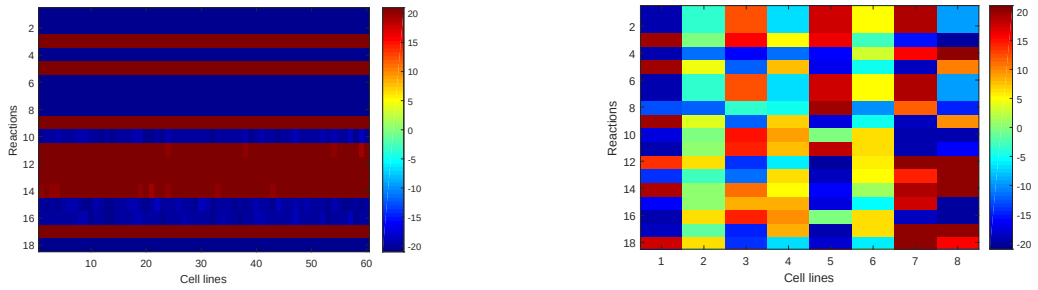
Fig. 4: Heat map of the flux distribution of the 3 number reactions in (A) cancer cell lines and (B) normal cell lines

Further, from the set of 74 number of reactions we found 18 reactions whose standard deviation of flux values in **the** normal cell lines are above a high value, say 12. These 18 reactions shows high flux values in all cancer cell lines but in normal cell lines the flux values are varying. The name of the reactions are given in Table 2.

Table 2: The reactions which show high flux in cancer cell lines but show fluctuations in normal cell lines.

acetyl-CoA transport, nuclear
Acetylcholin transport, nuclear trhough pores
L-ascorbate transport via facilitated diffusion
L-ascorbate transport via proton symport
Choline O-acetyltransferase(cytoplasmic)
Choline O-acetyltransferase' (nucleus)
Choline transport, nuclear through pores
citrate transport via sodium symport
coenzyme A transport, nuclear
cytidylate kinase (CMP,dGTP),nuclear
cytidylate kinase (dCMP,dGTP),nuclear
cytidylate kinase (dCMP),nuclear
bile acid intracellular transport
Na+ / iodide cotransport
nucleoside-diphosphate kinase (ATP:GDP), mitochondrial
nucleoside-diphosphate kinase (ATP:dGDP), nuclear
bile acid intracellular transport
UMP kinase (dGTP),nuclear

A heat map of the flux distribution of the 18 reactions in cancer cell lines is shown in Fig. 5A and for normal cell lines in Fig. 5B .



(a) Cancer cell lines (b) Normal cell lines

Fig. 5: Heat map of the flux distribution of the 18 number of reactions in (A) cancer cell lines and (B) normal cell lines.

4 Discussion

Cancer is a growing disease affecting a huge population. The existing treatments have lots of side effects. To minimize the side effects it is very important to systematically understand the mechanisms causing cancer. With the growing evidence that metabolism is perturbed in cancer [8], we used the existing metabolic models of cancer tissues and normal tissues and asked the question whether we can find reactions common across all cancer tissue types. Finding reactions with high flux across all cancer tissue types could be involved in cancer in all tissues or could just be normal tissues homeostasis mechanisms. For this, we analyzed the corresponding normal tissue metabolic networks and found reactions with high flux across cancer tissues and zero flux across normal tissues. These could possibly be involved with cancer in all tissue types.

The reactions we have found **during** the study could be important in causing cancer in a tissue dependent way or independent way and could be **the** hypothesis to be tested experimentally. We found some reactions with **continuous** high flux throughout cancer cell lines and **continuous** low flux throughout normal cell lines. They could be playing **an** important role in cancer. For example it is known that **cancer cells divide rapidly** and hence**, they** would need fatty acids for **their divisions; which is also mentioned** in [15–18] . Here also we observe acetyl-CoA C-acetyltransferase (ACAT) and fatty-acid-CoA ligase (ACSL)to be a important regulatory point in the cancer metabolic network, see Table 1. **This result is observed partially in different cancer cell line experiments. In a study [19], it was observed that ACAT1 is commonly up-regulated in diverse human leukemia, lung cancer, head and neck cancer, and prostate cancer cells in compare to corresponding normal cell type. There are studies that found that ACAT1, along with other ketogenic pathway enzymes, behaved functionally as a metabolic oncogene, as breast cancer cells over-expressing these enzymes had increased tumor growth and metastatic potential [20, 21]. There are clinical data which makes ACAT1 as potential prognosis marker for the pancreatic cancer [22]. There are literature which shows that other enzyme ACSL is also important in some cancer cells. ACSL showed to be over-expressed in colon and liver cancer cells [23, 24].**

On the other side, we would find some reactions show same behavior in all cancer tissue but varying behavior across normal tissue. However, we could not find any available literature would show the tissue specific behavior of the reactions. The reactions could be playing tissue specific roles. Our results could help in understanding in details the mechanistic insights into how a normal tissue becomes cancerous at metabolism level as well as how a normal tissue become cancerous faster than others.

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