Systems Biology Understanding of the Effects of Lithium on Cancer.

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Abstract

Lithium has many widely varying biochemical and phenomenological effects, suggesting that a systems biology approach is required to understand its action. Multiple lines of evidence point to lithium as a significant factor in development of cancer, showing that understanding lithium action is of high importance. In this paper we undertake first steps towards a systems approach by analyzing mutual enrichment between the interactomes of lithium-sensitive enzymes and the pathways associated with cancer. This work integrates information from two important databases, STRING and KEGG pathways. We find that for the majority of cancer pathways the mutual enrichment is many times greater than chance, reinforcing previous lines of evidence that lithium is an important influence on cancer.

Keywords

Lithium, systems biology, biochemical pathways, biochemical networks,

Introduction

Clinical and Epidemiological Context for Lithium and Cancer: By far the most common medical use of lithium is as a first line therapy for bipolar disorder, including associated depression as well as mania.[1] A comprehensive review of the literature confirms that lithium is also effective against unipolar depression with unique anti-suicidal effectiveness, and may also be useful against cancer and neurodegenerative disease.[2]

One line of evidence for the possible use of lithium as an anticancer agent is epidemiological. A retrospective study showed that psychiatric patients undergoing lithium therapy for bipolar disorder had a much lower incidence of cancer than a matched group not receiving lithium therapy.[3] More recent studies of similar design, one conducted nationwide across Sweden, and another across Taiwan, achieved the same result.[4 5] On the other hand another nationwide study, this time from Denmark, showed no correlation of lithium with colorectal adenocarcinoma.[6] On closer look, the Denmark study does not contradict the Swedish study. The Swedish study also found that for the entire population lithium was not correlated with cancer incidence, but in addition found that bipolar individuals not treated with lithium had a higher incidence of cancer than the general population. Lithium-treated bipolar patients, on the other hand, had essentially the same cancer incidence as the general population.

One piece of experimental evidence for lithium's potential as a cancer therapy is that inhibition of GSK3 was observed to inhibit prostate cancer cell proliferation[7]. A detailed study of molecular mechanisms by which lithium inhibition of GSK3B inhibits proliferation of prostate tumor cells in culture was presented by Sun et al.[8] The work was subsequently extended to an animal model.[9] A clinical trial for the

effect of lithium coupled with prostatectomy on men has been conducted but as of this writing results have not yet been published.[10]

With respect to other cancers, lithium has been found to be lethal to neuroblastoma cells but not to normal nerve cells.[11] The experimentally determined effective dose was 12 mM, a level which would be lethal if achieved systemically in a human or model organism but perhaps could be induced locally. A similar effect was found in ovarian cancer cells,[12] although a subsequent similar study on ovarian cancer cells suggests only a more modest benefit.[13] It is not clear from our reading of the two ovarian cancer papers why the results are significantly different from each other.

With respect to colorectal cancer, it has been found that lithium inhibits proliferation of a colorectal cancer cell line.[14] PTEN overexpression and lithium administration were shown to cooperatively inhibit proliferation of colorectal cancer cells.[15] Another study on colon cancer cells showed that lithium specifically induced a reversal of the epithelial-to-mesenchymal transition characteristic of the cancer cells.[16]

Two studies with relatively small sample size suggested a possible link between lithium and tumors of the upper urinary tract.[17] [18] However a large-scale study involving all urinary tract cancers in Denmark over a multi-year period found no correlation with lithium use.[19]

Because lithium therapy is systemic rather than topical or local, it follows that lithium might inhibit metastasis. Evidence that this is the case for colon cancer comes from observation of inhibition of metastasis-inducing factors by lithium and by observation on reduced metastasis in model animals given lithium therapy.[20]

Autophagy is a key cellular process in the inhibition of cancer.[21] Lithium has been shown to induce autophagy, due to its inhibition of inositol monophosphatase.[22] The full range of lithium effects on autophagy is complicated,[23] as might be expected because of lithium's lack of specificity.[2]

Because of the promising indications as cited above, lithium has been suggested as one of a number of drugs commonly used for other reasons, to be repurposed for cancer.[24]

Biochemical Context for Lithium and Cancer: Much of lithium's biochemical action may be summarized by noting that it inhibits, or competes with, action of magnesium.[2] Examples of such competition, that appear specially relevant for cancer, are given below.

Lithium appears to inhibit β-adrenergic and muscarinic receptor coupling to G proteins by competing with magnesium, which facilitates such coupling. [25] [26] [27] [28] [29] Overexpression of G protein coupled receptors is common theme of many cancers,[30] suggesting that inhibition of receptor-G protein coupling may inhibit cancer progression.

A particularly important example is substitution of a lithium ion for a magnesium ion acting as a cofactor in inositol monophosphatase, mentioned earlier in this paper in connection with autophagy. In this protein the binding site for lithium is not revealed in crystallography nor in solution NMR but can be identified in magic angle spinning solid state NMR, which is more suitable for systems with large internal motion.[31]

One mode of action with many consequences is lithium inhibition of glycogen synthase kinase 3 beta (GSK3B), mentioned earlier in this paper in connection with prostate cancer. Lithium inhibition of GSK3B was initially shown in vitro and in intact

cells,[32] and in the context of embryonic development.[33] It was later shown that lithium exerted its inhibitory effect on GSK3B by competing with magnesium for an essential binding site.[34] There are two closely related forms of GSK3, termed alpha (GSK3A) and beta (GSK3B), which are equivalently inhibited by lithium.[35] The two forms of GSK3 have substantial functional redundancy.[36] However some of their physiological properties are different, as demonstrated by the fact that GSK3B knockout mice are not viable,[37] but GSK3A knockout mice survive.[38] The very widespread nature of GSK3B effects is related to the large number of transcription factors that it regulates.[39] It functionally modulates cellular threshold for apoptosis,[40] it is central to mediating mitochondrial response to stress;[41] it facilitates immune responses by enabling the nuclear export of NF-ATc;[42] it regulates inflammation;[43] it regulates cardiac hypertrophy and development,[44] to name just a few. Based on microarray studies of brain cells in animals, lithium alters gene expression patterns significantly,[45] to be expected due to the large number of transcription factors regulated by GSK3B. Mice heterozygous for GSK3B exhibit similar behavioral traits to wild type littermates treated with 1mM lithium (a concentration that inhibits about 25% of GSK3 activity, in line with 1 of the 4 alleles of GSK3 inactivated in the GSK3B heterozygous mice)[46]

In addition to inhibiting the activity of GSK3B, lithium also inhibits its transcription.[47] Of all kinases, GSK3 appears to have the largest number of known substrates, over 100 known[48] and about 500 predicted by theory based on scanning and interpreting relevant motif sequences in the human genome.[49] Lithium will thus to some extent modulate activity along all pathways containing the hundreds of GSK3 substrates. So far, to our knowledge there are no published counterexamples to the hypothesis that lithium will exert an inhibitory effect on all proteins with essential magnesium binding sites, of which there are estimated to be over three thousand.[50]

Multiple other studies, in addition to those mentioned above, have addressed the potential efficacy of GSK3 inhibition as a therapeutic strategy against cancer.[51 52 53 54 55 56 57 58 59 60 61 62 63]

A widespread mechanism of lithium action is as a modulator of magnesium action in interacting with phosphate groups. The primary energy source for cells and the substrate for phosphorylating enzymes is not bare ATP, but rather magnesium-associated ATP (MgATP).[64] NMR studies show that lithium associates with MgATP.[65] Based on this admittedly small amount of data, we hypothesize that lithium associates with all magnesium-phosphate complexes and will thus modulate to some extent all phosphorylation reactions and all ATP-splitting processes. This is a reasonable interpretation of early work by Willis and Fang, in which lithium was found to increase the activity of the sodium-potassium pump without itself being transported significantly.[66] We have noted earlier in this paper the inhibitory effect of lithium on GSK3 by the mechanism of competing with Mg. Here we note that lithium also inhibits the activity of GSK3 by a second method, that is, by increasing phosphorylation.[67] Depending on context of relevant protein-protein interactions, lithium's effect on phosphorylation of a particular protein may be to either increase it or decrease it. For example, lithium decreases phosphorylation of tau-protein, presumably because it inhibits GSK3B, which is implicated in the phosphorylation of the tau-protein.[68]

Because lithium affects many different biological molecules and processes[2], it is essential to utilize the tools of systems biology[69] if a comprehensive understanding of

lithium action and its prospects for therapy are to be obtained. Important concepts for organizing biological information in a systems context are pathways and networks. A very useful tool for obtaining data about known pathways is the KEGG database.[70] An equally useful and complementary tool is the STRING database of interacting proteins.[71] In the present paper we investigate further the possible linkages among 1) lithium, 2) affective disorders, and 3) neurodegenerative disorders by analyzing the mutual enrichment between STRING-derived interactomes of lithium-sensitive enzymes, and the KEGG pathways associated with cancer.

Methods

Analysis was performed on the interactomes of lithium-sensitive genes, as identified by prior literature search[2]. This search suggested BDNF, BPNT1, DISC1, DIXDC1, FBP1, FBP2, GSK3A, GSK3B, inositol monophosphatases (IMPA1, IMPA2, and IMPAD1), INPP1, and PGM1 as key to understanding the broad biological actions of lithium. The interactomes of these genes were extracted from the STRING database (https://string-db.org). For each key gene, we adjust confidence level and order of neighbors (nearest only or next nearest included), so that each set contains a few hundred genes. This size is large enough for statistically reliable enrichment analysis. Very similar sets were merged; in particular FBP1 and FBP2 were merged into one set, and the inositol monophosphatases were merged into one set. On the other hand, GSK3A and GSK3B showed sufficient differences to be considered separately. Overall, we consider 10 distinct lithium-sensitive entities.

Disease Association

We used the R-package KEGGgraph[72 73] to identify the genes associated with the pathways of interest.

P-value calculation

The fundamental question we address is whether there is significant overlap or mutual enrichment between the interactomes of lithium-sensitive genes and the pathways or gene sets implicated in various cancers.

For each of the 10 lithium sets, an ensemble of 1000 null sets are generated by random selection from the human genome. Each null set is the same size as the corresponding lithium set. Then we used the R-package STRINGdb[74] to perform KEGG pathway enrichment analysis. This operation is a particular example of the powerful technique of gene-annotation enrichment analysis.[75] In gene-annotation enrichment analysis a test list of genes (often derived from gene expression experiments) is compared to an organized database of gene annotations, often referred to as a gene ontology[76], an array of gene lists corresponding to different biological functions, molecular functions, or locations in the cell. The output of the gene-annotation enrichment analysis is expressed as the likelihood that the list overlaps could have occurred by chance (p-value). A very low p-value implies that the degree of overlap is highly significant statistically and very likely is significant biologically. In our study the gene lists we are comparing are the interactomes of

lithium sensitive enzymes on the one hand, and KEGG pathways and Kegg pathways associated with cancer on the other hand. For each KEGG term retrieved, a null distribution of uncorrected *p*-value is generated by the 1000 null sets. This gives us a measure of the false discovery rate, since any overlap between the null sets and the KEGG pathways is purely accidentally. Then the fraction of null set uncorrected *p*-values smaller than or equal to the lithium-sensitive set uncorrected *p*-value would be the empirical *p*-value. For a detailed discussion of empirical p-value determination see Ge et al[77].

Key Gene Prediction

We predict key genes by counting how many times a gene appears in the cross section of interactomes and pathways associated with a particular disease. In this way, we predict which genes might be most important in disease-related pathways. Then, the genes are scored by the sum of mean counts over all diseases. A higher ranking indicates a gene would be associated with an important factor in many diseases.

Results

Fig.1 shows mutual lithium interactome enrichment with specific cancer pathways, represented by heatmaps. Each area on the heatmap is a color-coded representation of the degree of mutual enrichment between the genes in the interactome of the indicated lithium sensitive enzyme and the genes in the indicated pathway. The darker the shade, the more significant the mutual enrichment of the interactome-pathway combination is. The light areas on the heatmap represent situations where a lithium-sensitive interactome has little or no mutual enrichment with a cancer pathway. The dark areas, deep orange and red, represent situations where enrichment is very strong—far greater than could be expected by chance. Three genes stand out as being not strongly connected to cancer pathways: BPNT1, DISC1, and PGM1. Of the cancer pathways, breast cancer stands out as being not likely to be strongly influenced by lithium levels. For the remainder of the genes and the remainder of the cancers, the relationship between the lithium-sensitive interactome and the cancer phenome is strong. For example, for the DIXDC1 interactome, the strong correlation we see with cancer pathways is reinforced by data suggesting DIXDC1 implication in colon cancer,[78] lung cancer,[79] gastric cancer,[80] prostate cancer,[81] glioma,[82] and pancreatic cancer.[83] It should be noted that DIXDC1 has not been noted in the literature to be directly inhibited by lithium. Rather its modulation by lithium is a consequence of its proximity to GSK3B in functional protein-protein networks. Consequently, there is a significant overlap between its interactome and that of GSK3B. However, there is sufficient difference to warrant the two interactomes being considered as separate entities for purposes of the analysis presented here.

Figure

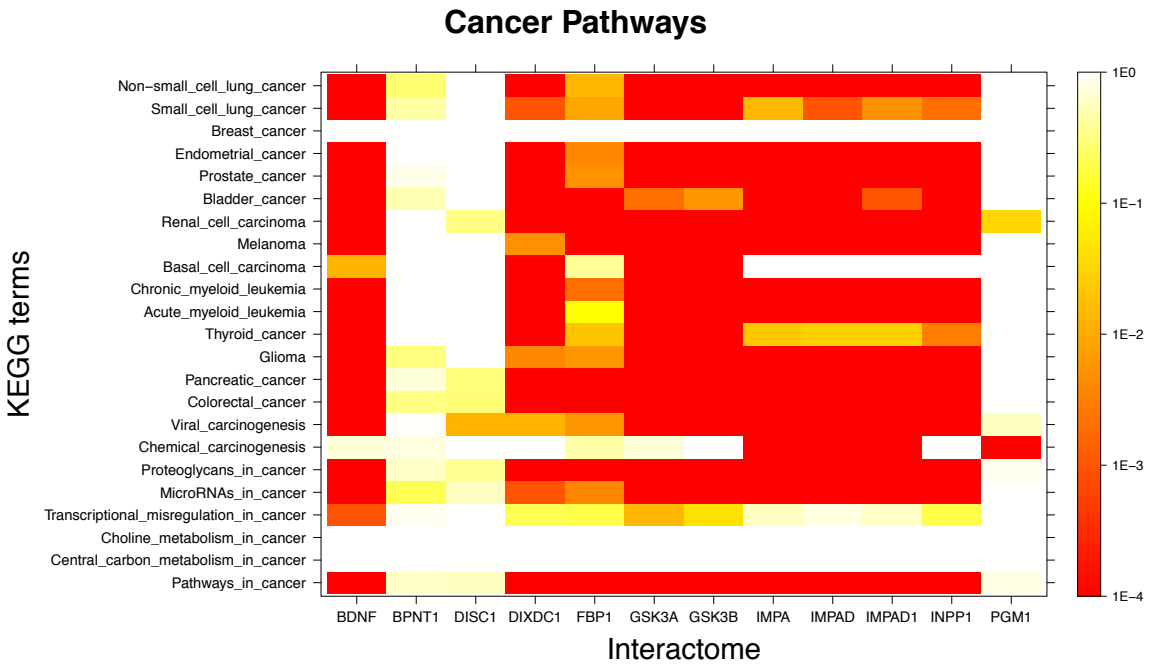

Figure 1. *Visual representation of mutual enrichment patterns between cancer-associated pathways and the interactomes of lithium-sensitive gene products. Calibration of p-value vs. color is indicated by a vertical scale to the right of the heat map. Red or dark orange indicates very strong enrichment while lighter color indicates weak or, if white, no enrichment. Three genes stand out as being not strongly connected to cancer pathways: BPNT1, DISC1, and PGM1. Of the cancer pathways, breast cancer stands out as being not likely to be strongly influenced by lithium levels. For the remainder of the genes and the remainder of the cancers, the relationship between the lithium-sensitive interactome and the cancer phenome is strong.*

For each of the cancer-associated pathways we wished to compute a single number representing the relative likely sensitivity of the disease to lithium, in order to contribute to prioritizing which diseases are most likely to benefit from clinical trials with lithium. There is a significant literature on combining p-values,[84] with choices among methods depending on the detailed structure of the data. We adopt a relatively simple approach, which is to compute the geometric mean of the individual p-values for each pathway-interactome mutual enrichment value.

$$p_{mean}=(p_1 \text{ x } p_2 \text{ x } p_3 \text{ x} \ldots\ldots p_n)^{1/n} \qquad \text{Equation (1)}$$

The method of averaging in Equation (1) ensures that both strong and weak enrichments contribute significant weight to the mean. Note that all of the p-values that go into Equation (1) are corrected for false discovery rate by random resampling. Thus, no further false discovery rate correction is necessary for computing p_{mean}. Note also that our method is bounded at the low end of p-values by the number of null samples it is reasonable to compute, given compute time constraints. For one thousand null sets as used in this paper, the computed p-value will be zero when none of the thousand null sets

shows the degree of enrichment of the test sets. For purposes of computing the p_{mean} in equation (1) we substitute 10^{-4} for zero for each of these cases.

Cancer-related KEGG pathway	p_{mean}	$1/p_{mean}$
Colorectal_cancer	0.00049903	2003.89209
Pancreatic_cancer	0.00053973	1852.76828
Proteoglycans_in_cancer	0.00054323	1840.83131
Renal_cell_carcinoma	0.00056283	1776.72794
Pathways_in_cancer	0.00056569	1767.76501
Chronic_myeloid_leukemia	0.00085134	1174.61894
Non-small_cell_lung_cancer	0.00090896	1100.15513
Endometrial_cancer	0.00091244	1095.95823
Prostate_cancer	0.00091481	1093.12212
MicroRNAs_in_cancer	0.00093106	1074.04618
Melanoma	0.00093303	1071.77346
Viral_carcinogenesis	0.00099762	1002.38273
Glioma	0.00122264	817.904951
Acute_myeloid_leukemia	0.00125638	795.934615
Bladder_cancer	0.00150566	664.158631
Small_cell_lung_cancer	0.00352993	283.291551
Thyroid_cancer	0.00567253	176.288152
Basal_cell_carcinoma	0.03711938	26.9401078
Chemical_carcinogenesis	0.05277248	18.9492716
Transcriptional_misregulation_in_cancer	0.14953148	6.68755483
Central_carbon_metabolism_in_cancer	1	1
Choline_metabolism_in_cancer	1	1
Breast_cancer	1	1

Table 1. *Rank order of significance of enrichment between lithium-sensitive interactomes and KEGG cancer-associated pathways. It is seen that for the great majority of pathways the mutual enrichment is very significant, with p-values significantly below .01. Breast cancer is unusual; it appears there is no enrichment beyond chance. The table also displays a "lithium sensitivity index", which is* $1/p_{mean}$

Table 1 shows in rank order the significance of enrichment between lithium-sensitive interactomes and KEGG cancer-associated pathways. It is seen that for the great majority of pathways the mutual enrichment is very significant, with p-values significantly below .01 Breast cancer is unusual; it appears there is no enrichment beyond chance. The table also displays a "lithium sensitivity index", which is $1/p_{mean}$ We should note that sensitivity to lithium does not necessarily imply a *beneficial* sensitivity. There are some indications for some cancers that lithium might be beneficial, as described in the Introduction section of this paper, but because of the complexity of the feedback relationships in these pathways, a complicated relationship between lithium ingestion and cancer incidence is very possible.

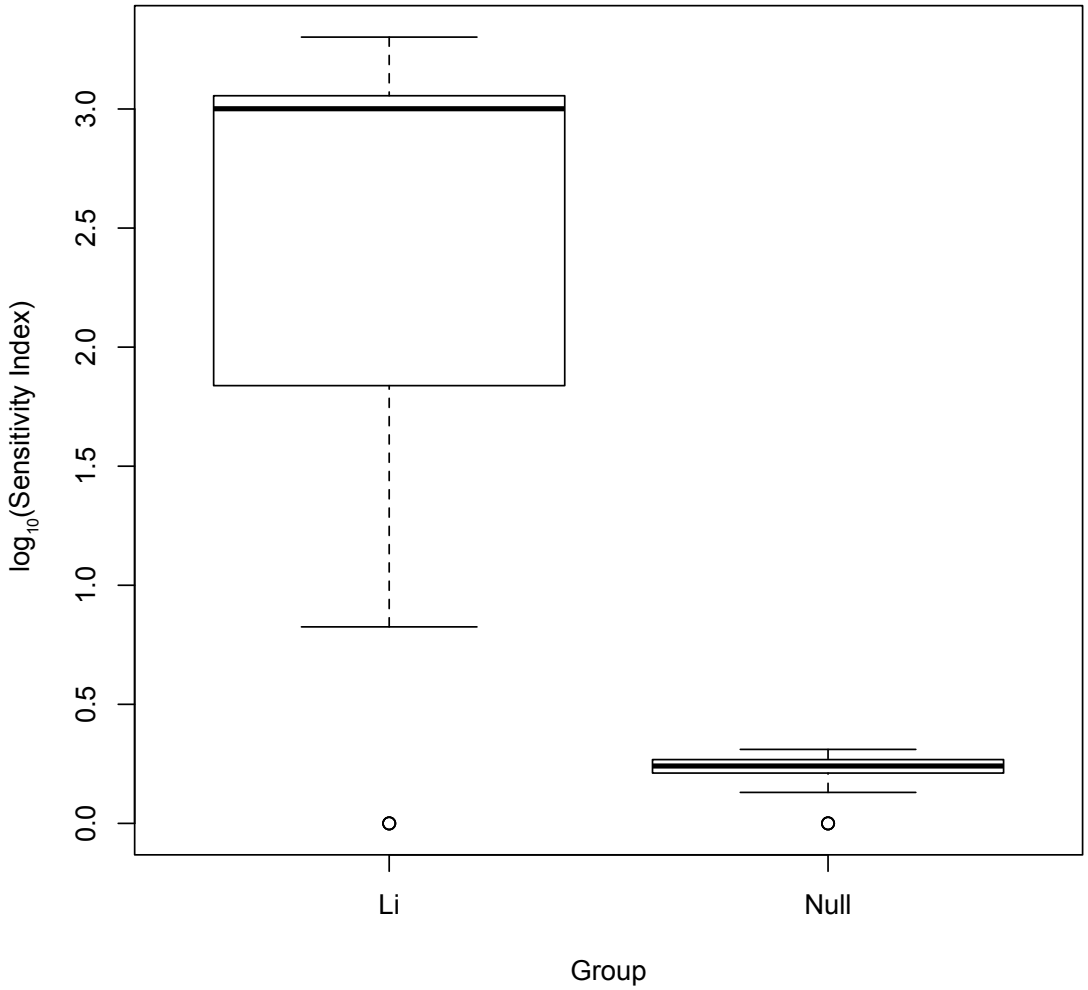

Figure 2. *This figure visualizes the strength of the projected lithium influence on cancer pathways. The logarithms of the lithium sensitivity indices ($1/p_{mean}$) are shown in boxplot format together with the corresponding results when the lithium interactomes are replaced with random gene sets. Essentially this figure shows the signal-to-noise ratio of our results and demonstrates conclusively that lithium ingestion is overwhelmingly likely to modulate the incidence of a wide range of cancers.*

Figure 2 visualizes the strength of the projected lithium influence on cancer pathways. In this figure the logarithms of the lithium sensitivity indices ($1/p_{mean}$) are shown in boxplot format together with the corresponding results when the lithium interactomes are replaced with random gene sets. Essentially this figure shows the signal-to-noise ratio of our results and suggests that lithium ingestion is overwhelmingly likely to influence the incidence of a wide range of cancers.

Summary and Discussion

We have conducted a pathway and network analysis exploring the role of lithium in multiple cancers. The results show that for the large majority of such cancers, there is high mutual enrichment between the interactomes of lithium-sensitive enzymes and the pathways associated with those diseases, indicating that lithium is very likely to affect the incidence and course of the disease. Our results are consistent with a variety of lines of evidence from both epidemiology and from experiment, cited in the Introduction section of this paper, suggesting possible influence of lithium on the incidence and progression of cancer.

We hope that the results described in this paper will contribute to prioritizing and designing clinical trials of lithium for cancer. To provide context for such prioritization and design, it is essential to take into account the ways in which lithium is unique, both as a pharmaceutical and as an ion that is ubiquitous in the environment, and therefore ubiquitous in the water and food we ingest[2]:

1. Unlike other ions, lithium is not regulated by selective membrane transport processes. Therefore, lithium concentration in both extracellular and intracellular compartments, rather than being roughly constant, is roughly proportional to lithium ingestion.
2. Unlike other pharmaceuticals, lithium is wildly nonselective in its biochemical effects. The major underlying mechanism for the lack of selectivity is lithium's general propensity to modulate the many biochemical processes and structures that involve magnesium.
3. Unlike other pharmaceuticals, lithium is an essential nutrient. The question with lithium is not whether it should be ingested or not, but rather how much. Extreme lithium deprivation results in failure to thrive, while too much lithium is toxic.

In the light of all these factors, we suggest that the correct question to ask with respect to lithium and a particular disease is not, "Should lithium be administered for this particular disease?" but rather, "What is the optimum blood level of lithium for this individual, given his or her disease history, status, genetic propensities, and other medications?" Unlike other pharmaceuticals that are far more specific and inhibit or activate one gene or a small number of genes, the model for lithium action is that it alters the balance between a large number of interacting processes and pathways. Thus, a dose-response curve for lithium is likely to be highly nonlinear and not always monotonic.

There are just a few well-established markers for optimum concentrations. For a patient with a reliable diagnosis of bipolar disorder a common target for optimality would be blood concentration of 0.8-1 mM. Significantly higher concentrations will result in acute toxicity, while significantly lower will result in loss of effectiveness. However, this level has some side effects when sustained for years or decades, namely an increased risk of kidney damage and lowered thyroid activity.

At the other end of the dosage scale, epidemiological evidence is compelling that geographical variations in concentration of lithium in the drinking water are correlated with a variety of health and wellness markers.

Another important marker is provided by a study showing that over a four-year period a lithium level of .25-.4 mM of lithium (1/4 to 1/2 of the bipolar therapeutic dose)

329 did not incur any renal damage[85]. This study suggests that clinical studies exploring low
330 to medium-dose lithium could be undertaken with relatively minimal concerns for side
331 effects.
332 Li et al have suggested GSK3B inhibitors for prostate cancer management.[86] One
333 possible piece of low-hanging fruit for a clinical trial would be low- to medium-dose
334 lithium, or other GSK3B inhibitors, for men undergoing active surveillance (AS) for
335 advance of prostate cancer. From studies of AS outcomes, a large fraction of patients on
336 AS ultimately require invasive treatment, as reviewed by Dall'Era et al[87]. When this
337 need arises it typically comes after only a few years. Thus, a trial of lithium in this
338 context would produce statistically significant results in a short time and, especially for
339 lithium, would be relatively inexpensive. One of us (EJ) conducted an informal one-
340 person trial on himself after being diagnosed with prostate cancer in 2014, ingesting
341 lithium supplements sufficient to bring his blood lithium to .3-.4mM while undergoing
342 AS by Memorial Sloan Kettering Cancer Center. (MSK did not prescribe the lithium but
343 agreed to include lithium level measurement in periodic blood tests.) In October 2017 EJ
344 was told that there was no longer a need for AS, as his markers were not progressing.
345 One case, important as it is to EJ, does not have statistical significance. We need clinical
346 trials with significant numbers of people.
347 We will be happy to collaborate on further specific pathway or network analysis
348 relevant to any of the cancers for which lithium, or other more specific drugs targeting
349 lithium-sensitive genes, may be a promising component of therapy.

350 **Author Contributions**

351 The work was planned jointly in conversations between EJ and WG. WG did the
352 computations and prepared the figures and tables. WG wrote the first draft of the
353 Methods and Results sections. EJ wrote the first draft of the Introduction and
354 Conclusions sections. Both authors shared in the final refinement of the manuscript.

355 **Conflict of Interest**

356 The authors have no personal, professional, or financial relationships that could
357 be construed as a conflict of interest with the work described in this paper.
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