Author’s response to reviews

Title: Elucidating mechanistic insights into drug action for atopic dermatitis: a systems biology approach

Authors:

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Author’s response to reviews:

Dear Dr. Tu,

We thank you and the reviewers for taking time to evaluate our manuscript titled “Elucidating mechanistic insights into drug action for atopic dermatitis: a systems biology approach”.

We went through the comments from reviewers on this manuscript, and have addressed them in the revised manuscript being submitted. Below, we provide details of reviewer’s comments and the revisions made in the manuscript to address these comments.

Reviewer 1

Comment-1.1 to the Author:

1. 117: Could you justify why "2-fold change" was used, and explain how this affects to the results (sensitivity analysis)?

Response from authors:

We used “2-fold change” (i.e. Log2 1) as a conservative cutoff to ensure that only those genes are considered that show at least 2-fold increase or decrease in expression as compared to the control (i.e. samples before topical treatment in our study). Based on your suggestion, we have also performed sensitivity analysis to understand the effect of fold change cutoff on the results. As detailed in Additional files 2, 3 and 4, the key pathways contributing towards the action of the drugs are captured by all the fold change cutoffs evaluated during our sensitivity analysis.

Comment-1.2 to the Author:
1. 122: "statistical methods" need more detailed information or appropriate references to be cited. For example, how was the variability among the 10 patients treated in the statistical analysis?

Response from authors:

Thank you for this suggestion. We have revised the section titled “Pathway enrichment analysis” under “Methods” in the manuscript by adding more details on the statistical methods. To address the variability among 10 patients, we have considered only those pathways for analysis that were observed to be enriched in more than 50% of the samples.

Comment-1.3 to the Author:

1. 131: Could you add some explanation of what "Gene Expression Overlay feature" is?

Response from authors:

Thank you for this suggestion. We have revised the section titled “Pathway enrichment analysis” under “Methods” of the manuscript to address this point.

Comment-1.4 to the Author:

Table 1:

* "Plays important role" - could this expression be a bit more elaborated?

* It may be easier for the readers to have 2 separate Tables: one for the pathways affected by BM and another for PC.

Response from authors:

Thank you for this suggestion. We have made two separate tables, one for the pathways affected by BM (see Table 1) and another for PC (see Table 2). Moreover, we have elaborated the roles of the genes and pathways mentioned in these tables.

Comment-1.5 to the Author:

1. 322: "established" sounds too strong, given that the results are based on the data from only 10 patients. Could you comment on generalizability of the results, or replace "established" by "suggested"?

Response from authors:
As per your suggestion on replacing “established” by “suggested”, we have made necessary revision in the manuscript.

Comment-1.6 to the Author:

Fig 3a: Too complex and too detailed - is there a way to simplify to make the message clearer? This comment is applicable to all the figures taken from eSkIN screen shot.

Response from authors:

We have revised all the figures showing eSkIN screenshots. Also, we have included a zoomed-in view of the important biomolecules and their interactions in Figures 3, 4 and 6 for better clarity.

Comment-1.7 to the Author:

Fig 3b: NfkB is unaffected for 8 out of 10 patients. Could you add some explanations on how Fig 3b demonstrates the effects of deactivation of NfkB?

Response from authors:

We wish to emphasize that although NfkB is significantly down regulated only in 2 out of 10 samples, it is indeed down regulated in all the other samples albeit in lower magnitude. We have revised the section titles “BM mediates its anti-inflammatory effect via TNF, TLRs and IL4 pathways” under “Results” in the manuscript to convey our approach for selection of NfkB as one of the down regulated genes.

Reviewer 2

Comment-2.1 to the Author:

Essentially this is a comprehensive analysis of the effect of BM and PC on eczema skin using this eSkin platform. No confirmatory or mechanistic analyses are performed. Neither is there comparison with other much more widely used analysis platforms. There would need to be analysis of the same data with another platform to demonstrate the increased utility of this highly curated dataset, and that it is not missing key pathways and genes important to skin function.

Response from authors:

Thank you for this suggestion. We have now performed the analysis with DAVID (https://david.ncifcrf.gov/), one of the most widely used tool for pathway enrichment analysis. We used two different annotation datasets, namely GO_BP_FAT and KEGG, to perform this analysis (see the section titled “Comparison of eSkIN results with DAVID” under “Results” for details). GO_BP_FAT was selected as it offers one of the most comprehensive mapping of genes
and their associated biological processes. However, it does not provide details of the molecular interactions among the genes (or their protein products) in the biological processes. To address this, we also performed the analysis using KEGG as the annotation dataset that provides details on molecular interactions in the pathways.

The results obtained from DAVID using GO_BP_FAT and KEGG and the comparison with the results obtained from eSkIN is presented in the section “Comparison of eSkIN results with DAVID” under “Results”. As is evident from Table 3, eSkIN is able to identify all the relevant pathways that are observed to be enriched in DAVID analysis (using GO_BP_FAT as annotation dataset). This corroborates the capability of eSkIN to perform skin-centric pathway enrichment analysis. On the other hand, the enriched pathways from DAVID analysis using KEGG as the annotation dataset are very generic and could not be interpreted in the context of their relevance to skin physiology.

It is also important to note that eSkIN allows further exploration of the enriched pathways to understand molecular interactions among the genes (or their protein products) of interest, which is not available for the pathways obtained from DAVID (using GO_BP_FAT dataset). Although the pathways obtained from DAVID analysis using KEGG dataset allows exploration of molecular interactions, the enriched pathways themselves were not found to be relevant in the context of skin physiology.

**Comment-2.2 to the Author:**

All the findings are anecdotal as presented, and even what seems to me the clearest result, epidermal barrier proteins up-regulated with PC, down regulated with BM is not backed up statistically. There need to be thorough statistical analysis to identify key markers and pathways.

**Response from authors:**

As per our understanding, the reviewer comments on following aspects of our study: (1) novelty of our findings, and (2) statistical analysis.

**Novelty of our findings:**

Although the biological pathways and phenotypic effects of the drugs are reported earlier, the molecular markers and their functional roles in actuating the effects of these drugs were unavailable. In this study, we used a computational systems biology approach to identify these molecular markers and derive insights into their mechanistic role towards drug action. We have revised Tables 1 and 2 of the manuscript to detail the findings from our study. An example of one such novel finding is in Keratinocyte Differentiation pathway, that highlights an alternate route via CD44 and AKT1 that could lead to down regulation of the expression of skin barrier proteins in BM treated samples, thus, affecting barrier function. Moreover, based on our analysis, we have proposed insights into the manifestation of disease during drug treatment that were not reported earlier.
Statistical analysis:

We wish to emphasize that the identification of enriched pathways on treatment with BM and PC is based on the pathway enrichment analysis performed using eSkIN. As elaborated in the section titled “Pathway enrichment analysis”, pathway enrichment analysis relies on Fisher’s exact test to compute the probability of a pathway being enriched based on the formula shown in Equation 1. Only those pathways enriched with p-value < 0.05 were considered for further study, thus, ensuring that statistically significant pathways are analyzed. Using this set of statistically significant enriched pathways, the key markers were identified based on their expression profiles. Similar approaches have been used in earlier reports to gain insight into functional roles of differentially expressed genes (Zhao et al. International Journal of Endocrinology (2015) Article ID 164087; and Wu et al. Journal of Integrative Bioinformatics (2016) 9:213). We have revised the section titled “Pathway enrichment analysis” under “Methods” of the manuscript to elaborate on the statistical analysis performed in our study.

With the suggestions of the reviewers incorporated in the revised manuscript, we hope that the reviewers would now find the manuscript suitable for publication in BMC Dermatology. If there are any further suggestions from reviewers, we would be happy to address them.

Sincerely,

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