Author's response to reviews

Title: Fibroblasts from phenotypically normal palmar fascia exhibit molecular profiles highly similar to fibroblasts from active disease in Dupuytren's Contracture

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Author's response to reviews: see over
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Dr. Ashani Weeraratna
Associate Editor
BMC Medical Genomics

Re: MS: 1611516212570843

Satish et al., “Fibroblasts from Phenotypically Normal Palmar Fascia Exhibit Molecular Profiles Highly Similar to Fibroblasts from Active Disease in Dupuytren’s Contracture”

Dear Dr. Weeraratna,

We were pleased to receive your letter of September 23, 2011 expressing interest in the findings detailed in the manuscript referenced above. We are now pleased to submit the revised version of the manuscript incorporating the changes requested by the Reviewers. Enclosed please find the final revised version of the manuscript which addresses the concerns of the Reviewers with the necessary emendations incorporated. This letter addresses the specific critiques of the Reviewers in turn; the Reviewers’ comments are italicized and are followed by our response in plain text.

Concerns of Reviewer 1:

Major concerns:

Obtained results are presented reasonably and are of interest to researchers or clinicians with closely related research interests. Even though the methods employed are up to date and represent standard methodology, it should be stated that neither one validation nor functional analysis has been carried out upon microarray results analyses. The authors should note that statements on clinical relevance or functional implications should be validated with at least one of widely accepted methods (e.g. quantitative reverse transcription-PCR for chosen genes. In vitro models, analysis of targeted miRNAs etc.-please refer to just few randomly chosen articles dealing with microarray studies as a guideline: PLoS one 6 (2011) Tsuge et al.; Am J Transplant. (2011) doi: 10. 1111 Scian et al.; Genomics 97 (2011) De Felice et al.). Indeed, the authors have not succeeded to propose some novel mechanistic interpretation for Dupuytren’s contracture pathogenesis due to lack of validation data. They presented a variety of possible cellular processes represented by cluster of expressed genes in Tables 1 and 2 that might be of certain interest. Again, validation data would certainly help in filtering only those processes that are really relevant to disease development or predisposition. For the reader is rather difficult to comprehensively read and interpret the data presented in Tables 1 and 2 as too many possible pathways of common knowledge (more than 30) related even to other specific disorders (e.g. ovarian cancer signaling, acute myeloid leukemia signaling, amyotrophic lateral sclerosis signaling etc.) have been presented.

In conclusion, authors are encouraged to perform validation/functional studies and to compare obtained results not only with their own results but to critically discuss recent papers not only with their own results but to critically discuss recent papers related to the topic (e.g. data speaking in favor of intrinsically similar characteristic of DC-derived cells from affected and unaffected tissues and some novel, validated signaling pathways implicated in DC have already
been reported in literature but are not mentioned by the authors. These data would certainly improve the discussion section).

We appreciate the efforts of this Reviewer in thoroughly reviewing this submission and offering valuable insights towards achieving a compelling revised manuscript. We appreciate that the Reviewer acknowledges that “results are presented reasonably and are of considerable interest,” and that “the methods employed are up to date and represent standard methodology.” The Reviewer’s main concern was that no validation of the microarray data was offered, and s/he suggested we perform quantitative real time RT-PCR to confirm said data. In addressing this issue we emphasize that we observed remarkable internal consistency within each different tissue type between six unrelated individuals, which is essentially itself a cross validation of the data.

Nevertheless, as recommended by the Reviewer, we did also directly confirm microarray data in three instances using real time RT-PCR. We chose to study ANGPTL7, LAMA5 and SHROOM2 as these are genes which have not been reported previously to be differentially expressed in Dupuytren’s cord contracture but which have potential mechanistic implications that may be relevant to Dupuytren’s. We note that in all three cases the qRT-PCR results conformed extremely well with the microarray results, lending confidence that the array data as a whole is similarly accurate.

The signaling pathway analysis that we have presented was obtained through Ingenuity Pathway Analysis which does, in this case, result in a significant number of identified networks. We realized on review that we had included a group of networks in table 2 that were of marginal significance as they had scores of <3. These networks have been removed from this submission, and thus we present only 12 networks in Table 2. We hope the reviewer will agree that restricting this data will be less confusing to readers, while still presenting the data in as comprehensive a fashion as possible.

Also, we have significantly expanded the Discussion section to address both this Reviewer’s comments as well as Reviewer 2’s comments, and hope the Reviewers will find these additions useful.

-Major Compulsory Revisions

Pursuing on the presented study in more details, which include at least validation of most interesting data obtained in microarray assays, would ensure the scientific impact and clinical relevance of presented data.

As noted above, we have followed the reviewer’s recommendations and have performed quantitative real time RT-PCR on 3 candidate genes (see above) to validate the microarray data.

-Minor Essential Revisions

More comprehensive presentation of data

We have attempted to ensure that the data is presented comprehensively for easy understanding, but again note that Table 2 now focuses on a more restricted but presumably also more significant group of identified networks.

-Discretionary Revisions
Authors are encouraged to compare obtained results not only with their own results but to critically discuss recent papers related to the topic (e.g. data speaking in favour of intrinsically similar characteristic of DC-derived cells from affected and unaffected tissues and some novel, validated signaling pathways implicated in DC have already been reported in literature but are not mentioned by the authors. These data would certainly improve the discussion section).

We have expanded the Discussion section significantly in this regard as suggested by the Reviewer.

Concerns of Reviewer 2:
1. The manuscript is generally well written and organized.
2. It does ask scientific questions relevant to translational medicine.

We thank the Reviewer for these comments.

3. The study design is good. However, it is not clear to me that patients undergoing Carpal Tunnel Surgery are great controls. The results would be more impressive and pertinent if truly normal controls were used. However, getting surgical samples from healthy subjects is not as easy and feasible proposition, and the question asked with this approach (ie using the CT samples) also has the potential to answer distinct and relevant questions.

We agree with the Reviewer that normal palmar fascia from completely disease-free hands would be the best control, but this is not practical to obtain. We do believe, and believe the data set from this paper proves, that using CT samples as a control does allow us “to answer distinct and relevant questions,” and specifically address the question of relevant controls in the Discussion.

In the present study we have not used just CT’s as our controls we have also utilized unaffected palmar fascia from Dupuytren’s patients as another control. We have explicitly addressed the issue of using different controls in the Discussion part of the manuscript which we would like to mention it here. “If patients with a genetic predisposition to develop DC have inherited PF cells that exhibit profound differences in gene expression to normal palmar fascia (CT) cells, then PF cells represent the most clinically relevant controls for testing treatments designed to prevent DC progression. In contrast, normal palmar fascia cells, such as CT cells, are valuable for analyses designed to identify the molecular characteristics that distinguish DC cells from normal cells”. As the reviewer correctly acknowledges obtaining surgical samples from healthy subjects is not easy and feasible.

4. The microarray study seems methodologically sound and the statistics seem robust.

We appreciate the Reviewer’s kind words.

5. The findings support that there is a genetic predisposition to aberrant proliferation in the DC patient fibroblasts even though they appear phenotypically normal. This leads to the proposition that pharmacological treatment to prevent recurrence may be possible in those patients who have already developed DC, if targets are identified.

We agree with the reviewer’s summary of the findings of the manuscript.
6. The pathway analysis discussion to be the area where this manuscript could be improved. The discussion is vague and does not explore potential treatment targets. I would have liked to see an improved discussion about the role of β-catenin and more informative explanation of identified signaling pathways.

We have added more commentary about the potential role of beta-catenin in the Discussion, as the Reviewer suggests. We have also expanded the Discussion to comment on the three validatory gene targets we assayed to confirm the microarray data. Obviously, it is not possible to comment on every pathway or network identified in a microarray experiment, and hope the Reviewer agrees that the Discussion is now of greater range and utility to the reader.

We hope that we have addressed all of the Reviewers’ concerns and we appreciate the efforts of the Reviewers and believe their recommendations have strengthened our submission. We hope the Reviewers and Editors will find our amendments satisfactory.

Thank you once again for the opportunity to publish our work in your journal.

Very truly yours,

Latha Satish PhD