Reviewer's report

Title: Angelica sinensis promotes myotube hypertrophy through PI3K/Akt/mTOR pathway

Version: 1 Date: 9 December 2013

Reviewer: Matthew Alexander

Reviewer's report:

The research article by Yeh and colleagues describes the characterization of Angelica sinensis (AS) (commonly referred to as dong quai), an herbal medicine used by athletes and the general population to improve athletic performance and overall stamina. The authors look directly at its functional role in muscle cells in cell culture, with an emphasis on a potential link between AS and myotube hypertrophy. The authors identified ferulic acid as being the main potent compound of AS via HPLC chromatography, and go on to characterize the expression levels of PI3K/AKT/mTOR signaling factors in C2C12 myoblasts/myotubes treated with AS and a series of PI3K/AKT/mTOR pathway inhibitors and/or activators.

Major Essential Revisions:

1. My main scientific criticism of this manuscript is the fact that the authors state that the PI3K/AKT/mTOR pathway is affected by AS treatment of myotubes, yet the authors look at AKT and mTOR, yet not other aspects of this signaling pathway. Were the PI3K complex (p85, p110) components and or PTEN expression levels detected? Were the downstream signaling factors of PI3K/AKT/mTOR signaling pathway detected and quantified, namely 4E-BP1/phospho-4E-BP1 and/or S6K? The authors should look at the downstream signaling factors at either the RNA or protein levels following AS treatment of myotubes if they want to fully support their claim that AS is having its hypertrophic effects in myotubes directly through this pathway.

2. I had an overall difficult time in reading this manuscript. Throughout the manuscript there are terms that are confusing to the reader (ex. proliferous property, page 5, line 78; do the authors mean promote cell proliferation?). I strongly recommend the authors either have a native English speaker or carefully edit the manuscript for English grammar and sentence comprehension and clarity.

Minor Essential Revisions:

1. The HPLC chromatogram is only discussed in the Methods section and not the Results. If the authors do not want to discuss this in the Results section, then they should move it to the Supplemental Data/Figures.

2. The myotube pictures in Figure 2 appear green tinted and are difficult to view and interpret. The authors should either adjust the filter settings on the images or
retake the pictures for clarity.

3. In the Methods the authors cite another paper for the description of how the differentiated myoblasts into myotubes, the authors should specifically state how (reduced serum percentage) and when they differentiated the myoblasts into myotubes in the Methods.

4. Figure 4A typo, “Relativ” on y-axis change to “Relative”.

5. Figure 4 and 5 western blots. Given that some of the differences in blot band intensities appear to be minor, the authors should include a loading control. There is a reference to a Beta-actin antibody from Sigma used, but I don’t see it in the manuscript. Also, this is a minor point but the authors should be consistent in their references to certain companies (Cell Signaling Technology in Danvers, MA, USA or Beverly, MA?). Just one of the Cell Signaling Tech company citation is needed.

6. Minor comment, the authors state that they used “fresh growth medium” for the “NON” samples. There’s some discrepancy, was this growth or differentiation medium? The authors need to be specific and state this in the Methods or at very least the Figure legends.

7. References/citations are needed on page 5, sentences on lines 66, 69.

8. pg17, line 295 Awkward sentence.

Discretionary Revisions:
None.

**Level of interest:** An article of importance in its field

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

I declare that I have no competing interests.