Author's response to reviews

Title: Nck2 promotes human melanoma cell proliferation, migration and invasion in vitro and primary melanoma-derived tumor growth in vivo

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Author's response to reviews: see over
September 13, 2011

Prof Calvin Roskelley
Associate Editor, BMC Cancer
BioMed Central

Dear Prof Roskelley,

Please find included a revised version of our manuscript: "Nck2 promotes human melanoma cell proliferation, migration and invasion in vitro and primary melanoma-derived tumor growth in vivo" (MS: 177517132578527). In this revised version, we have restructured the abstract as recommended and addressed the Reviewers’ comments. All these changes were properly underlined to facilitate your revision. We also included below a point-by-point response to the Reviewers’ concerns that we hope will be satisfactory.

I would like to thank you and the additional reviewer for expert advice and consideration.

Sincerely yours,

Louise Larose Ph.D.
Associate Professor
Email: louise.larose@mcgill.ca
Point by point Reply to Reviewers’ Comments

Reviewer #1 (Dr. Takeshi Senga)

*It would be better if the authors examine whether suppression of Nck2 inhibits migration or invasion of invasive melanoma cells.*

In this study, we demonstrated that overexpression of Nck2 in human primary melanoma that rarely metastasis is sufficient to induce metastatic characteristics such as increased proliferation, migration and invasion. According to our data showing that in human primary melanoma overexpressing Nck2, cell proliferation, migration and invasion are still lower than in metastatic melanoma expressing similar high levels of Nck2, we cannot exclude that other yet identified players could be required to fully promote metastasis in melanoma overexpressing Nck2. None the less, we agree with the reviewer that addressing whether suppression of Nck2 inhibits proliferation, migration or invasion of invasive melanoma cells would be of great interest to further define the role of Nck2 in metastasis. In this study, we showed that depletion of Nck2 in invasive melanoma cells using transient transfection of Nck2 siRNA inhibits cell proliferation (Figure 4). However, low efficacy of transient transfection or short duration of sufficient inhibition of Nck2 expression by siRNA did not allow proper investigation of migration or invasion using this approach. We believe that stable cell lines established using Nck2 shRNA from plasmid transfection or virus infection will be more appropriate to evaluate whether Nck2 depletion inhibits migration or invasion of metastatic melanoma cells. To date, we did not succeed to inhibit Nck2 expression in invasive melanoma using stable transfection of a commercial Nck2 shRNA plasmid. Further investigation considering other Nck2 shRNA and methods of delivery (retrovirus) are under progress in our laboratory. Once established, we will address the role of Nck2 in invasive metastatic melanoma as suggested by the reviewer.

Reviewer #2 (Dr. Calvin Roskelly)

*The authors should refrain from stating, as they do in the discussion, that their investigation has been focuses on determining the mechanism responsible by which overexpressed Nck2 contributes to tumor progression.*

As recommended by the reviewer, we have modified this statement in the abstract and the discussion of our revised version of the manuscript (changes were underlined).

*To consider discussing the question of whether Nck2 overexpression is necessary for melanoma progression given that all their experimental gain of function data point towards sufficiency.*

As discussed above (reply to Reviewer #1), we demonstrated that increased expression of Nck2 in human primary melanoma that rarely metastasis is sufficient to promote metastatic
characteristics. However, our study did not address whether Nck2 is necessary for melanoma metastasis. None the less, we provided some insights suggesting that Nck2 could play such function. In fact, we found higher levels of Nck2 expression in metastatic compared to non metastatic cell lines in three different types of cancer. In addition, we demonstrated that depletion of Nck2 in metastatic melanoma reduces cell proliferation. Moreover, as discussed above, our data do not exclude that other players could contribute to fully induce progression of melanoma overexpressing Nck2 towards a metastatic phenotype. This point has been further considered in the discussion of the revised version of our manuscript (changes were underlined).