Reviewer's report

Title: Overexpression of Hepatoma-Derived Growth Factor in melanocytes does not lead to oncogenic transformation

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Reviewer: Ming-Hong Tai

Reviewer's report:

Summary
Elevated hepatoma-derived growth factor (HDGF) expression has been identified as a poor prognostic marker in a wide range of cancer including melanomas. In this study, Sedlmaier et al. generated transgenic mice overexpressing HDGF in melanocytes (HDGF-Tg) to investigate whether HDGF act as an oncogene and directly transform melanocytes in vivo. However, these HDGF-Tg mice did not exhibit increased incidence of melanoma nor skin abnormalities. To further confirm the oncogenic role of HDGF, the authors crossed the HDGF-Tg and HDGF-null with mice carrying deficiency with Ink4a tumor suppressor gene, then challenged with UV light to increase the risk for melanoma development. Unexpectedly, only HDGF-/-/Ink4a+/-, but not HDGF-Tg/Ink4a+/- mice displayed an increased number of premelanocytic alterations with no melanomas detected. The authors thus concluded that HDGF has no direct role on cellular transforming in melanocytes, but rather plays a role of secondary hit or non-oncogene addiction in tumor progression. Despite of extensive and tremendous work in raising various kinds of transgenic animals and skin histological analysis, further validating studies are required to confirm this contradictory finding.

Major Compulsory Revisions:

1) The authors showed no evidence of HDGF overexpression in melanocytes of their HDGF-Tg mice. The immunofluorescence analysis in Fig. 2A and in situ hybridization in the supplementary Fig. 1 are not convincing. The authors should perform immunoblot analysis of HDGF protein level in skin of HDGF-Tg mice as the basal HDGF level is detectable in skin from wild type mice based on their immunoblot results in Fig. 2C-a (1st lane). Besides, RT-PCR analysis of unique HDGF transcript level in skin from Tg, wild type, and HDGF-null mice would assist to solve this critical issues.

2) In Fig. 2B, the authors observed an interesting phenomenon that primary melanocytes isolated from HDGF-Tg mice, but not wild type or HDGF-null mice, could not survive in vitro, then proposed that excessive HDGF in the skin of HDGF-Tg mice hampered the maturation or differentiation process, ultimately leading to the death of primary melanocytes. Such argument would be strengthened by monitoring the viability of primary melanocytes from wild type and HDGF-null mice after exogenous supply of recombinant HDGF or ecotropic
HDGF gene transfer. Alternatively, the authors could investigate the viability of primary melanocytes from HDGF-Tg/HDGF-/- mice (as they used in Fig. 1A) to evaluate whether such deficits could be rescued by decreasing HDGF gene doses.

3) In the Discussion (page 11, line 20), the authors wrote “Enomoto and coworkers recently reported on the investigation of a albumin-HDGF transgenic mouse model targeting HDGF-overexpression to hepatocytes (Enomoto H 2009), In this case the overexpression of HDGF resulted in delayed hepatocyte maturation, suggesting that HDGF-overexpression in hepatocytes partially suppresses hepatocyte differentiation”. After reviewing the cited reference (Enomoto H et al. Hepatology Research 2009; 39: 988–997), there was no description of such albumin-HDGF transgenic mouse model. Please clarify the source of such citation.

Minor Essential Revisions:
1. proof read the manuscript text before submission.

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

**Quality of written English:** Acceptable

**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’