Reviewer’s report

Title: Truncated LEF-1 is one of the key regulators in the growth of colon carcinoma

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Reviewer: Andrew Quest

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Revision of manuscript entitled “Truncated LEF-1 is one of the key regulators in the growth of colon carcinoma” by Wang et al

LEF-1 is a transcription factor implicated in the progression of colon cancer. A truncated form referred to here as LEF-1-dL also exists, which might act as an inhibitor of LEF-1 function. Alternatively, evidence is available indicating that the two forms potentially cooperate in the control of cellular function. In colon cancer cells an increase in the expression of full-length LEF (LEF-1-FL) is observed while LEF-1-dL presence is reduced. Here the authors first show that alterations in the presence of these two isoforms are observed in colon samples from patients. Specifically, increases in LEF-1-dL were detected in normal and tumor-adjacent tissue while LEF-1-FL prevailed in tumor samples. Subsequently, the role of these different isoforms was studied in the two colon carcinoma cell lines HT29 and SW480. Overexpression of LEF-1-dL coincided with reduced proliferation and increased cell death in vitro. Moreover, expression of LEF-1-dL reduced migration in response to SDF-1 of HT29 cells in a transwell assay. This was linked to reduced expression of the chemokine receptor CXCR4. Finally, tumor growth in vivo, as well as vacularization were reduced in tumors formed by LEF-1-dL expressing SW480 and HT29 cells. Taken together these results indicate that variations in the expression of these two LEF-1 variants have a substantial impact on the behavior of colon cancer cells.

In general the experiments are well done and the results obtained support the conclusions. However, some issues need to be addressed.

Major comments

In the methods section it is not specifically stated how many patient samples were obtained and analyzed. Was the total number evaluated 22 as stated in the results section? Additionally, the authors mention obtaining tumor samples and adjacent normal tissue. Yet in the results section (see Figure 1) three types of tissue samples were evaluated, colon carcinomas, adjacent tissue AND normal tissue. It is not clear from the information available what exactly the samples analyzed represent. This needs to be clarified.

Moreover, in Figure 1, are the Western blots of the three tissue samples from two or more patients? Presumably these are representative results. The authors
should indicate in each case, how many they are representative of. The authors should indicate in all these panels where appropriate molecular weight markers migrate.

In Figure 2, cells are compared after analysis of the cell cycle by flow cytometry. Rather surprisingly, no increments in the sub G0/G1 population are detected for both the SW480-dL and HT29-dL cells. This would be expected based on the result shown in Figure 3 where an increase in Annexin-V positive cells is observed for both these cell lines. The authors need to provide an explanation for this discrepancy and preferably should include the percentage cells in sub G0/G1 in the analysis shown in Figure 2.

In Figure 4, the authors talk about a colony forming assay in panels A-D. In is not clear from the description provided what exactly the authors are measuring here. Is this anchorage-independent growth in a colony forming assay? This needs to be explained better in all relevant sections. In panels B and D, the y-axis should probably be labeled “Colony numbers” rather than “Clone numbers”.

In Figure 5, the authors analyze expression of their his-tagged constructs in tumor-derived cells. From the methods section, it is not clear how these cells were obtained. Moreover, all tagged proteins shown are of the same size. According to the results in Figure 1, LEF-1-dL is notably smaller. The authors need to explain better what is being demonstrated. Also molecular weight markers should be included.

In Figure 6, tumor growth is documented for the different HT29 and SW480 variants. In Figure 7, vascularization of tumors is characterized using different markers in one set of tumors. However, the information provided is confusing. In the figure panels, HT29 is mentioned while the figure legend states that tumors derived from both HT29 and SW480 cells were analyzed. This needs clarification. Also, the authors should evaluate whether reduced tumor vascularization observed for cells expressing the LEF-1-dL variant is also significantly reduced in tumors of the same size. The current analysis detects differences in tumors of different sizes.

The conclusion at the end of the discussion is that “the balance between the two forms of LEF-1 might have important consequences for normal growth of colon cancer cells and cancer”. This statement doesn’t really coincide with the message provided by the title “Truncated LEF-1 is one key regulator in the growth of colon carcinoma”. The title needs to be altered to reflect better the findings of the paper.

The English of the manuscript needs major revision preferably by a native speaker. There are a number of confusing statements throughout the text. For instance, at the end of the second paragraph in the results section, the authors summarize by saying “These results indicate that LEF-1 may play an important role in colon carcinogenesis”. The statement follows just after a section describing how HT29 and SW480 cells expressing the different LEF-1 variants were generated. Thus, it would appear out of place.

Minor comments
Figure 1. should be HeLa rather than Hella.
There are many typo errors throughout the text
Throughout the text several abbreviations are not explained (CSFE, SDF-1, CXCR4, MMPs, CDK etc). Also is the abbreviation Hif-1a or HIF-1a?

**Level of interest:** An article whose findings are important to those with closely related research interests

**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