Annals of Hepatology

LIVER NEWS ELSEWHERE

March-April, Vol. 13 No. 2, 2014: 305-307

SALLuting a new biomarker in hepatocellular carcinoma

Andrea L. Inghilesi, Fabio Marra

Dipartimento di Medicina Sperimentale e Clinica, University of Florence, Florence, Italy.

Article commented:

Yong KJ, Gao C, Lim JS, Yan B, Yang H, Dimitrov T, Kawasaki A, et al. Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. *N Engl J Med* 2013; 368: 2266-76.

Comment:

Hepatocellular carcinoma (HCC) is the sixth most prevalent cancer worldwide and the third most frequent cause of cancer-related death, with increasing incidence in traditionally low-frequency regions like the United States and Northern Europe.[1] A relevant proportion of patients with HCC present with advanced disease, and the only approved therapy in this subgroup is the multi-kinase inhibitor sorafenib, approved in 2008.[2] The lack of novel and/or second-line therapies, and the limited survival benefit provided by sorafenib underscore the urgent need for novel treatment options in advanced HCC. This goal may be more easily achieved with a better understanding of the molecular pathogenesis of this cancer, which is characterized by a marked heterogeneity. Unfortunately, the molecular mechanisms leading to HCC diversity and to its variable prognosis are still far to be defined.

The paper by Yong, *et al.*, recently published in *The New England Journal of Medicine*,[3] describes the possible role of SALL4 as a biomarker of aggressive hepatocellular carcinoma and a possible novel target for therapy in HCC. SALL4 (Sal-like 4) is one of the human homologues of the homeotic gene spalt (*Sal*) in Drosophila, and encodes for a zinc-finger transcription factor. SALL4 has a key role in the maintenance of pluripotency and self-renewal of embryonic stem cells,[4-6] and mutations in this gene are associated with the Okihiro syndrome, characterized by multiple organ defects and a phenotype similar to thalidomide embryopathy.[7] SALL4 has been previously associated with regulation of liver development. Its expression has been described in fetal hepatoblasts but not in adult hepatocytes, and expression levels of SALL4 gradually fall during liver maturation.[8] In adulthood, SALL4 expression has been reported in various types of cancer[9-11] and may play a significant role in their development.

Yong, *et al*. constructed a panel of tissue microarrays consisting of 179 surgically resected HCC, and matched, archived, non-neoplastic liver specimens obtained from the National University Hospital of Singapore, and compared the differential expression of SALL4. Immunohistochemical analysis showed a significantly increase in the number of cells with SALL4 expression in HCC specimens compared to non-neoplastic tissue. 55.6% of HCC showed SALL4 positivity, although the levels of expression were variable. These findings were validated in an independent group of specimens obtained from 228 patients in Hong Kong, and analyzing data from public gene expression databases. The Authors next established whether expression of SALL4 had a prognostic role for patients with HCC. In both the Singapore and Hong Kong cohorts, a multivariate analysis identified SALL4 expression as an independent prognostic factor for overall survival (in both series) and early recurrence (only in the Hong Kong cohort).

In the past few years, transcriptional profiling of human HCC has identified several subgroups, including tumors with phenotypic features of stem cells or progenitor cells, which are particularly aggressive and associated with a poor prognosis.[12] In a tumor, cells with stem-cell properties are indicated as tumor-initiating cells or cancer stem cells, and are believed to play a pivotal role in initiation, maintenance and recurrence of different types of cancer,

Correspondence and reprint request: Fabio Marra, MD, PhD
Dipartimento di Medicina Sperimentale e Clinica
University of Florence
Largo Brambilla, 3. I50134 Florence, Italy
Phone: +390557945425. Fax: +39055417123
E-mail: fabio.marra@unifi.it

Manuscript recived: December 14, 2013.
Manuscript accepted: December 14, 2013.

including HCC.[13] Yong, *et al*. performed a hierarchical cluster analysis of gene expression from human primary hepatocytes, fetal liver tissue and HCC, showing that tumors with high SALL4 clustered with fetal livers, whereas low-SALL4 HCC clustered with normal hepatocytes. In addition, high-SALL4 tumors were enriched in genes upregulated in HCC with poor survival or with embryonic stem cell signatures. To provide more mechanistic data, SALL4 expression was knocked down in SNU-398 HCC cells with specific short hairpin RNA (shRNA). While gene expression of SALL4-knocked down cells clustered with human hepatocytes, SNU-398 cells treated with a control, scrambled shRNA clustered with human fetal liver tissue, further confirming the role of the SALL4 transcription factor in conferring stemness features. Moreover, SALL4 knockdown decreased cell viability and limited tumorigenesis in an experimental animal model.

Transcription factor activity is usually more difficult to be therapeutically targeted compared to protein kinases, which are effectively modulated by small molecule inhibitors. To demonstrate a potential translatability of their experimental observations, the authors used as a competitive inhibitor a recently described SALL4 12-AA peptide, that blocks the interaction between SALL4 and NuRD (nucleosome remodeling and histone deacetylase [HDAC] complex).[14] Interaction between SALL4 and NuRD results in downregulation of the tumor suppressor PTEN, which acts on the PI3-kinase/Akt pathway. When SNU-398 cells were treated with the SALL4 antagonistic peptide, a reduction of cell viability was observed, demonstrating the efficacy of this approach. Finally, the Authors tested the *in vivo* effects of the SALL4 12-AA peptide in a xenograft model of HCC in which SNU-398 cells had been transplanted subcutaneously. A significant reduction of tumor load was observed in mice treated with the antagonistic peptide compared to the group treated with a control peptide.

The paper by Yong, *et al*. is of great interest in the field of HCC for a number of reasons. SALL4 has received considerable attention in the past few years for its role as a determinant and marker of embryonic stem cells and of cancer stem cells in different tissues. Remarkably, the results of the study published in *The New England Journal of Medicine*[3] are in agreement with those of two other papers which have appeared in 2013. Oikawa, *et al*.[15] found expression of SALL4 in HCC, cholangiocarcinoma, and mixed forms of liver tumor, and showed that expression of this molecule is associated with poor prognosis and with increased expression of stem cell markers. Moreover, a Japanese group has recently reported that SALL4 is activated in a subtype of HCC with stem cell features, and again confirmed poor prognosis compared to SALL4-negative patients.[16] An intriguing finding from this study is the observation that SALL4-positive HCC is significantly associated not only with markers of hepatic stem cells but also with HBV infection.

One of the most interesting aspects emerging from the work of Yong, *et al*.[3] and from the other recent studies on this molecule[15,16] is the dual role of SALL4 as a biomarker and a potential new target for therapy. This latter aspect was analyzed in all three studies by combined *in vitro* and *in vivo* approaches, including an injectable 12-AA peptide. While a strategy directly targeting SALL4 deserves further analysis, elucidation of its downstream effectors could provide alternative ways to interfere with the aggressiveness of this subtype of cancers. SALL4 signals through NuRD, a complex associated with histone deacetylase activity, and histone deacetylase inhibitors are currently investigated in HCC. Similarly, the fact that SALL4 decreases PTEN and results in boosted activity of the PI3-kinase/Akt/mTOR pathway could pave the way to the use of specific related inhibitors[17] in patients with high SALL4 expression. In addition, in the Hong Kong cohort of the Yong study,[3] expression of SALL4 was associated with cancer recurrence. Along these lines, the observation that some SALL4 expression is detectable even in non-neoplastic tissue may contribute to explain the higher risk of risk of recurrence, as demonstrated for other gene signatures in the cirrhotic liver.[18]

An equally important aspect is the significance of SALL4 as a biomarker to detect cancers with a more aggressive phenotype, in line with other stem-cell gene expression profiles. Serum alpha-fetoprotein has been indicated as a marker of HCC with progenitor-like phenotype and poor prognosis,[12] and could potentially substitute for measurement of SALL4 in a liver biopsy. In the correspondence following publication of the SALL4 paper, Yong, *et al*. report that serum alpha-fetoprotein levels greater than 100 ng/mL can identify patients with high SALL4 expression in tumor tissue with sensitivity and specificity of 66.7 and 68.4%, respectively.[19] The diagnostic accuracy of the measurement of circulating SALL4 levels in HCC, as reported for breast cancer,[20] needs further evaluation in the near future.

A final remark that is inspired by this excellent study is the re-evaluation of the role of liver biopsy

for a more accurate stratification of the different subtypes of HCC and of the related prognosis, as shown by the comparison of SALL4-high and low HCC. HCC is one of the few solid tumors for which treatment is not guided by molecular characterization. The possibility to diagnose HCC in a cirrhotic liver based only on imaging features has certainly helped the clinical management of the patients, but has indirectly represented a hurdle to advance our knowledge in the molecular characterization of this tumor, and in the design of personalized treatments. This is clearly reflected in the recently published phase II study evaluating tivantinib in patients with HCC failing sorafenib treatment,[21] where drug-induced prolongation of the time-to-progression was markedly more evident in patients with high expression of c-met in tumor tissue. Studies like those describing the significance of SALL4 should encourage to obtain biopsies at least in the setting of controlled clinical trials.

REFERENCES

1. Forner A, Llovet JM, Bruix J. Hepatocellular carcinoma. *Lancet* 2012; 379: 1245-55.
2. Llovet JM, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, et al. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; 359: 378-90.
3. Yong KJ, Gao C, Lim JS, Yan B, Yang H, Dimitrov T, et al. Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. *N Engl J Med* 2013; 368: 2266-76.
4. Elling U, Klasen C, Eisenberger T, Anlag K, Treier M. Murine inner cell mass-derived lineages depend on Sall4 function. *Proceedings of the National Academy of Sciences of the United States of America* 2006; 103: 16319-24.
5. Yang J, Gao C, Chai L, Ma Y. A novel SALL4/OCT4 transcriptional feedback network for pluripotency of embryonic stem cells. *PloS one* 2010; 5: e10766.
6. Rao S, Zhen S, Roumiantsev S, McDonald LT, Yuan GC, Orkin SH. Differential roles of Sall4 isoforms in embryonic stem cell pluripotency. *Molecular and Cellular Biology* 2010; 30: 5364-80.
7. Kohlhase J, Chitayat D, Kotzot D, Ceylaner S, Froster UG, Fuchs S, et al. SALL4 mutations in Okihiro syndrome (Duane-radial ray syndrome), acro-renal-ocular syndrome, and related disorders. *Hum Mutat* 2005; 26: 176-83.
8. Oikawa T, Kamiya A, Kakinuma S, Zeniya M, Nishinakamura R, Tajiri H, et al. Sall4 regulates cell fate decision in fetal hepatic stem/progenitor cells. *Gastroenterology* 2009; 136: 1000-11.
9. Ma Y, Cui W, Yang J, Qu J, Di C, Amin HM, et al. SALL4, a novel oncogene, is constitutively expressed in human acute myeloid leukemia (AML) and induces AML in transgenic mice. *Blood* 2006; 108: 2726-35.
10. Cui W, Kong NR, Ma Y, Amin HM, Lai R, Chai L. Differential expression of the novel oncogene, SALL4, in lymphoma, plasma cell myeloma, and acute lymphoblastic leukemia. *Modern pathology: an official journal of the United States and Canadian Academy of Pathology, Inc* 2006;19: 1585-92.
11. Kobayashi D, Kuribayshi K, Tanaka M, Watanabe N. SALL4 is essential for cancer cell proliferation and is overexpressed at early clinical stages in breast cancer. *International Journal of Oncology* 2011; 38: 933-9.
12. Lee JS, Heo J, Libbrecht L, Chu IS, Kaposi-Novak P, Calvisi DF, et al. A novel prognostic subtype of human hepatocellular carcinoma derived from hepatic progenitor cells. *Nat Med* 2006; 12: 410-6.
13. Rountree CB, Mishra L, Willenbring H. Stem cells in liver diseases and cancer: recent advances on the path to new therapies. *Hepatology* 2012; 55: 298-306.
14. Gao C, Dimitrov T, Yong KJ, Tatetsu H, Jeong HW, Luo HR, et al. Targeting transcription factor SALL4 in acute myeloid leukemia by interrupting its interaction with an epigenetic complex. *Blood* 2013; 121: 1413-21.
15. Oikawa T, Kamiya A, Zeniya M, Chikada H, Hyuck AD, Yamazaki Y, et al. Sal-like protein 4 (SALL4), a stem cell biomarker in liver cancers. *Hepatology* 2013; 57: 1469-83.
16. Zeng SS, Yamashita T, Kondo M, Nio K, Hayashi T, Hara Y, et al. The transcription factor SALL4 regulates stemness of EpCAM-positive hepatocellular carcinoma. *J Hepatol* 2014; 60: 127-34.
17. Moeini A, Cornella H, Villanueva A. Emerging Signaling Pathways in Hepatocellular Carcinoma. *Liver Cancer* 2012; 1: 83-93.
18. Hoshida Y, Villanueva A, Kobayashi M, Peix J, Chiang DY, Camargo A, et al. Gene expression in fixed tissues and outcome in hepatocellular carcinoma. *N Engl J Med* 2008; 359: 1995-2004.
19. Yong KJ, Chai L, Tenen DG. Oncofetal gene SALL4 in aggressive hepatocellular carcinoma. *N Engl J Med* 2013; 369: 1171-2.
20. Panis C, Pizzatti L, Herrera AC, Cecchini R, Abdelhay E. Putative circulating markers of the early and advanced stages of breast cancer identified by high-resolution label-free proteomics. *Cancer Lett* 2013; 330: 57-66.
21. Santoro A, Rimassa L, Borbath I, Daniele B, Salvagni S, Van Laethem JL, et al. Tivantinib for second-line treatment of advanced hepatocellular carcinoma: a randomised, placebo-controlled phase 2 study. *Lancet Oncol* 2013; 14: 55-63.