Reviewer's report

Title: SOX2 Expression Correlates with Lymph-Node Metastases and Distant Spread in Colon Cancer

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Reviewer: Vittorio Colantuoni

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In this manuscript, Neumann et al. investigated a series of colorectal cancers (CRC) for the expression of SOX2 and beta-catenin, two important players in colon tumorigenesis by immunohistochemistry. Specifically, the simultaneous expression of both genes has not been investigated so far and was assessed to verify whether it may influence CRC progression, in particular node and distant metastases formation, and tumor outcome. SOX2, in addition, is a known marker of colon steamness. The expression of both genes is reported to be associated with higher rate of node and distant metastases resulting in a worse prognosis. The association between these two genes is an interesting topic but requires more experiments to be strengthened; some results are not completely analyzed and discussed, the conclusions drawn should be more detailed.

Major points:

1. Positive SOX2 nuclear immunostaining was detected only in 24 out of 114 patients (21.1%). In contrast, negative immunostaining was detected in 90 cases (78.9%), suggesting that the large majority of the cases analyzed are SOX2 negative. Node and liver metastases are more associated with the SOX2 positive tumors: thus, SOX2 seems to promote tumor spreading only in 20% of the cases analyzed, 7% of which are also beta-catenin positive. These results seem to weaken the initial assumption "both beta-catenin and SOX2 have a prognostic value for the occurrence of distant metastases". The association of SOX2 negative cases with tumour grade G2 and G3 (88 cases, 77.2%), node infiltration (both N0 and N1) and metastases (both M0 and M1), i.e. advanced tumour stages should be discussed.

2. Since no data on the SOX2 cytosolic staining are reported, it is assumed to be negative. This piece of data should be presented, even if negative. Similarly, no data are reported on SOX2 expression in the normal adjacent mucosa to compare the relative expression levels in both tissues. All these informations may be important in view of the fact that SOX2 is reported to be expressed also in the normal mucosa and changes in its expression levels in matched tissues could provide insights into the role played by this factor.

3. The cohort of selected patients was divided in two arms according to the presence or absence of synchronous liver metastases. Some characteristics of the two arms are reported only in the Methods section (tissue collection); more informations are required to correlate the SOX2 expression levels.
4. A high nuclear positivity for beta-catenin is reported in 76 patients (66.7%), although no association is found with node or liver metastases. In contrast, the 38 cases with low nuclear positivity (33.3%) show a strong significant association with liver metastases (27 M1 vs 11 M0 cases; 23.7 vs 9.6%) (p=0.001). Does the relative nuclear positivity correlate with a higher rate of liver metastases and tumor progression? How do these results reconcile with the statement that “60-80% of CRCs develop on the basis of a dysregulation of the Wnt/beta-catenin signalling pathway”? These questions should be addressed and would probably help in discriminating tumors with early dysregulation of the Wnt/beta-catenin pathway from those in which this alteration occurs later.

5. In the abstract and introduction the authors report that SOX2 can repress beta-catenin transcription activity. This topic should be discussed and, if available, data should be presented to support or dismiss this evidence.

6. “Combined high scores of SOX2 and nuclear beta-catenin expression were present in 8 of 114 cases (7%)… This subgroup showed significant correlations with nodal status and distant spread. Nevertheless, no correlation between SOX2 and nuclear or cytoplasmic beta-catenin was observed”. In light of these results, how can we state that “SOX2 protein expression in addition to beta-catenin may be a possible prognostic marker in the management of CRC patients”? 

7. SOX2 is reported as a colonocytes stemness marker. This statement could have a stronger impact if other stemness markers be investigated such as Oct3/4, KLF4 or, alternatively, CD133 and Lgr5. This would also clarify the relationship between their activation with that of the Wnt pathway in colorectal cancer stem cells.

8. It is known that other genes and pathways are responsible for initiation/progression of tumors localized at the right colon. It would be interesting to know whether SOX2 expression and/or beta-catenin levels correlate with CRC localization. These data may be relevant in shedding light into new alternative pathogenetic pathways.

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

Quality of written English: Needs some language corrections before being published

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'.