Reviewer’s report

Title: RKIP phosphorylation and STAT3 activation is inhibited by Oxaliplatin and Camptothecin and are associated with poor prognosis in Stage II colon cancer patients

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Reviewer: Fahd Al-mulla

Reviewer’s report:

The paper by et al., entitled “RKIP phosphorylation and STAT3 activation is inhibited by Oxaliplatin and Camptothecin and are associated with poor prognosis in Stage II colon cancer patients” describes an interesting observation that IL-6 induced STAT3 activation and phosphorylation of RKIP. co-treatment of HCT116 cells with IL-6 and 300 μM OXP for 18 hours inhibited the increase in pY705 STAT3 and pRKIP caused by IL-6. Similarly, Camptothecin reduces IL-6 induced RKIP phosphorylation and STAT3 transcription. Moreover, the levels of pRKIP were reduced after STAT3 overexpression . The authors then studied STAT3 and pRKIP expression in stage II CRC and correlate these with clinicopathological characteristics of the patients.

These are interesting data. However, this reviewer sees ampule room for improvement.

Both RKIP and pRKIP actions may be cell specific. The authors present data based on one single cell line, which really compromised these novel findings. Illustrating the same consequences in another cell line will strengthen their conclusions significantly.

As stated Chemotherapy induced the expression of RKIP. The authors did not comment on why RKIP expression in the Western blots did not increased after OXP or Camptothecin treatment? Moreover, if the RKIP antibody detects total RKIP (RKIP and pRKIP) why it is not elevated IN THE WESTERN BLOTS?

RKIP reduced or loss of expression has been well documented in a variety of cancers especially stage II CRC. The authors provided no data on this but rather focused their findings on p-RKIP, which (overexpression) had been previously shown to be associated with good prognosis not worse. Can the authors provide data on RKIP survival in stage II CRC?

This reviewer is concerned about the term ‘Limited’ used to designate the number of patients used in the survival calculation. What are the numbers? Also, the authors did not attempt a multivariate analysis on the dataset. This is extremely important to exclude other confounding factors associated with poor prognosis in stage II (Like T-Stage, which has not been mentioned, MMR-status etc)

It is not clear which p-RKIP antibody was used in the Immunohistochemistry (Although in the method section STAT3 antibody source was stated clearly, the
same was not true for pRKIP. If the same anybody was used for the Western blotting then there is a problem because the sensitivity and the specificity has not been determined for the pRKIP in immunohistochemistry. Santa Cruz pRKIP clone sc-32623 is a polyclonal antibody and in our hands proved problematic on immunohistochemistry. This reviewer suggests that the staining be performed again with monoclonal antibodies for RKIP and pRKIP on the same section (Epitomics).

This reviewer is also concerned that scoring of the sections appeared different for cytoplasmic and nuclear stains. For example, 0,+1-+2 were grouped together in cytoplasmic and designated low expression, while only 0 scores were designated as low in nuclear scoring. This is not appropriate and should be discouraged. Similarly, what happens when p-RKIP is +3 for both nuclear and cytoplasmic (Figure 4A)? These may be a totally different and important subclass?

Minor changes:
There are numerous grammatical and spelling errors that needs to be corrected

The introduction and discussion need to refer to recent papers showing the prognostic value of RKIP and lymphovascular invasion in stage II CRC (Raf kinase inhibitor protein expression combined with peritoneal involvement and lymphovascular invasion predicts prognosis in Dukes’ B colorectal cancer patients. Doyle B. et al) and the relationship between RKIP and chemotherapeutic resistance (A new model for raf kinase inhibitory protein induced chemotherapeutic resistance. Al-Mulla F, et al.)

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