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Title: INTERPLAY BETWEEN POST-TRANSLATIONAL COX-2 MODIFICATIONS AND THE METABOLIC AND PROTEOMIC PROFILE IN A COLORECTAL CANCER COHORT.

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2 Peer-review report

Reviewer #1: In the core tip: the authors state that the levels of both COX2 and PGE2 are elevated in tumor cells but later state that PGE2 levels are significantly lower in tumor cells. Please correct this. Thanks for the comment. We agree with the referee because this sentence can be confusing. We have corrected it in the new version of the manuscript.

In the introduction the following sentence is irrelevant as you are talking about diagnosis and then jump towards treatment "In order to improve the diagnosis tools, current research focuses on the molecular basis of the disease." Ok, we have deleted it.

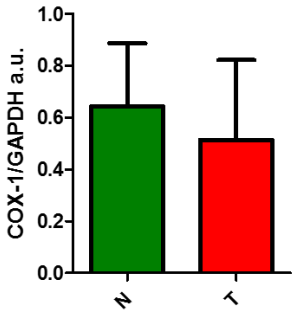
In the results section, it would be better to section it into subsections; for instance, relating to the proteomic and another to the metabolic profile. You can add a section in the discussion about how your results could affect diagnosis, prognosis or treatment strategies of CRC. Thanks for the suggestions. In the new version of the manuscript the results section has been sectioned according to the presented data. Moreover, we have added a part in discussion concerning the future strategies in CRC.

Minor comments: The manuscript needs some minor English and language editing. Below are some however there are many more throughout the manuscript, please check. Change "to" to "with" in the following sentence: proteolysis through the endoplasmic reticulum pathway and has been associated to alterations in the activity of the enzyme Change "and" to "an" in the following

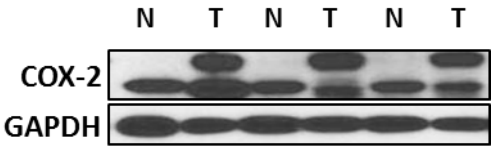
sentence: Nuclear magnetic resonance techniques (HRMAS) provide and in-depth analysis for the identification Change "in" to "on" in the following sentence: Although several recent studies have been carried out in CRC patients with NMR techniques, they have been mainly focused in the improvement of screening Change the following sentence "The mean of ages of the participant volunteers were" to this " the mean age of participants was". We apologize for the errors and we have corrected it in the new version. However, the manuscript has been revised by an English expert.

Reviewer #2: Title: New biomarkers in colorectal cancer: the paradigm of post-translational modifications of COX-2 Manuscript Type: Basic study The study of Prieto et al. study new biomarkers of colorectal cancer based on post-translational modifications of COX-2. They found an altered metabolic and proteomics profile that can be correlated to post-translational COX-2 modifications that induced decreased levels of PGE2. These findings could explain that, although COX-2 was initially proposed as a potential molecular predictor for CRC, its predictive relevance is controversial because other studies have been unable to identify a significant correlation between CRC patients outcome and COX-2 expression mainly due to the high variability in COX-2 levels among CRC patient's. The state-of-the-art is adequate to the field of research. The paper is original because they demonstrated the existence of metabolites that can modify the activity of this enzyme and that could be used as potential biomarkers in this type of cancer as well as the glycosylated-COX-2. However, some revisions should be done to increase the strength of the paper.

1. Previous research pointed COX-1 as an important molecular target for colorectal cancer (Li H et al., 6-C-(E-phenylethenyl)-naringenin suppresses colorectal cancer growth by inhibiting cyclooxygenase-1. Cancer Res. 2014 Jan 1;74(1):243-52 as an example). So this issue should be discussed in deep. Thanks for the comment. It is true that the inhibition of COX-1 can be effective in preventing several tumor types as colon cancer. Thus, in the manuscript of Li H et al, they suppressed tumor growth *in vitro* and *ex vivo* by inhibiting COX-1 activity with 6-CEPN. However, we did not detect any significant change in COX-1 levels neither in HT29 cells nor in tumor samples from CRC patients compared to normal mucosa (see graph below). Therefore, we think that the changes detected in PGE_2 levels cannot be associated to COX-1 changes in our cohort.

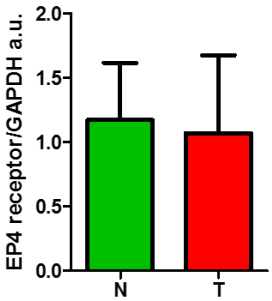


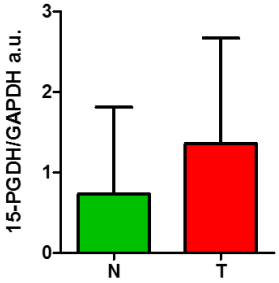
2. In results sections (Figure 1A,) please include more Normal/Tumour cases in the western blot. We consider that adding more normal/tumor cases in the figure, may complicate its understanding. However, we show below an example of western blot with several samples corresponding to different patients of CRC where you can see the dual COX-2 bands in the tumor tissue. We are confident on the quality of the Ab, since the upper band can be transformed into the lower band after deglycosylation.



Reviewer #3: I do not agree with the authors concerning their title: indeed, the relations existing between the progression of colorectal cancer and the role of COX-2 are very documented. We agree with the reviewer and we have changed the title in the new version.

Furthermore, I am surprised that the authors find a very low production of PGE2 in tumoral tissues versus non-tumoral tissues. It can be the reality of the study of their colorectal cancer cohort but the authors do not even discuss this surprising result. Indeed, it is known that it is the PGE2 which by settling on its target receptor (EP) who will be responsible for the tumoral proliferation. Thanks for the comment. Certainly, a higher PGE_2 production in tumors has been broadly described in the literature but surprisingly this is not the reality of our cohort where we have determined the levels of this mediator in 30 pairs of N/T samples detecting significantly less quantity of this PGE_2 in tumors. Furthermore, these levels were also significantly lower in tumors that only present the upper COX-2 band, corresponding to the glycosylated state of the protein. Indeed, if we analyze the downstream signaling of PGE_2, mainly mediated by EP4 receptors in CRC, we do not detect any significant change in tumors from our CRC cohort compared to normal mucosa (see left graph below). Furthermore, our samples presented high variability in 15-PGDH levels between patients with a tendency to be higher in tumors (see right graph below). Therefore, in our opinion, the low PGE_2 levels that we detected in tumors of our CRC cohort can be due, not only to post-translational modifications in COX-2 protein that may reduce its activity, but also to the presence of significant levels of 15-PGDH and to the absence of increased levels of EP4 receptors.





We have modified the figure 1 with additional new data and we have discussed this aspect more deeply in the discussion section of the new version of the manuscript.