Author's response to reviews

Title: Dissecting the signaling pathways associated with the oncogenic activity of MLK3 P252H mutation

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Author's response to reviews: see over
Dear Dr. Corso, Editor of the BMC Cancer,

Please find an electronic version of the manuscript that we would like to submit the Journal of Molecular Biology Research, entitled “Dissecting the signaling pathways associated with the oncogenic activity of MLK3 P252H mutation”.

We have previously reported the presence of functionally relevant MLK3 mutations in primary MSI gastrointestinal cancers. In particular, we have shown that cells expressing mutant forms of MLK3 exhibit increased transforming potential and were able to develop locally invasive tumors when subcutaneously injected in nude mice when compared to wild-type MLK3-expressing cells (Velho et al., Human Molecular Genetics, 2010).

In this work, we uncover the signaling pathways associated to the oncogenic effects of mutant MLK3. To this end, we performed expression microarray analyzes in cells expressing wild-type MLK3 and one of the most oncogenic mutations, the P252H. Between the wild-type and the mutant form of MLK3, a final set of 445 genes were differentially expressed. The genes identified were significantly enriched (p<0.05) in several KEGG pathways involved in overall biosynthesis processes, as well as in response in disease relevant processes. Interestingly, the colorectal pathway, which encompasses several relevant pathways such as WNT, MAPK, NOTCH, TGF-beta and P53, were significantly over-represented. Moreover, we verified that the expression of several components of the WNT/β-catenin pathway such as DVL2 and LEF1 as well as the canonic targets CCND1 and c-Myc were down-regulated in MLK3 mutant cells. In addition, the expression levels of well-known negative regulator of canonical WNT signaling, were up-regulated. On the other hand, FZD6 and FZD10 genes, known to act as negative regulators of the canonical WNT signaling cascade and as positive regulators of the planar cell polarity (PCP) pathway, a non-canonic WNT pathway, were found to be up-regulated in P252H cells. These results suggest that the canonic signaling of the WNT pathway is downregulated in P252H expressing cells, and the non-canonical WNT signaling, the PCP pathway, is in turn activated.
In terms of cellular effects, the present work sheds light on the molecular mechanism underlying the invasive potential of MLK3 mutant cells observed in our previous work. This effect is likely to be associated to the activation of Planar Cell Polarity pathway.

Overall, our results favor a functional role for mutant P252H MLK3 by deregulating signaling pathways known to play important roles in the development and progression of colorectal cancer leading to an aggressive behavior. Our results suggest that mutant MLK3 leads to the activation of the non-canonical WNT signaling pathway as the Planar Cell Polarity signaling and in this way induces migration and invasion of cancer cells.

All authors revised the last version of the manuscript and agreed with its submission to BMC Cancer, and it is not under submission in any other journal.

Looking forward to hearing from you,

Professor Raquel Seruca  
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Vice-President of IPATIMUP