Review of: "Integrated analysis reveals FOXA1 and Ku70/Ku80 as direct targets of ivermectin in prostate cancer"

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The research reported the direct targets of ivermectin in prostate cancer, cytosolic FOXA1 and NHEJ repair executer Ku70/Ku80, which explained the effect of Ivermectin on cell cycle, apoptosis and DNA damage or DNA damage repair. Methodically, integrated omics profiling, including RNA-seq and thermal proteome profiling were appropriately applied. The results highly indicated that IVM might be a promising chemotherapeutic strategy for prostate cancer, especially AR+ PCa. It was very clear that IVM inhibited the proliferation of PCa cells and induced DSB and apoptosis of PCa cells. Moreover, it was relatively clear that IVM inhibited the AR signaling pathway and downregulated the expression of E2F1 target genes. However, the results and conclusions from CESTA and TPP-TR would require further verification and discussion. The details as follows:

1. The CESTA is a new method to identify the drug targets in vivo that has been developed since 2013. Can it detect weak or transient interactions? Please comment.
2. Considering that IVM can cause ROS elevation, and ROS may have a general influences, including inhibition of E2F activity, DNA damage, are ROS -associated proteins involved in the IVM/TPP-TR candidates? If involved, would it be better that the author compares it with the reported mechanism in the present study, namely the direct binding of FOXA1 or Ku70/Ku80. If it is not involved, is it possible that it is due to the sensitivity of the method?Could ROS mechanism be preliminarily excluded or verified ?
3. FOXA1 and Ku70/Ku80 are the direct binding target molecules of IVM. Can other methods be used to verify their interaction, such as BioAcore, ITC or other methods?
4. IVM is a macrolide drug, are there the characteristics of its bound proteins? What is the subcellular localization of the differential proteins identified by TPP-TR, cytoplasm, plasma membrane or nucleus? Might other classes of macrolides have anti-prostate cancer effects? Please comment.
5. In the KEGG analysis, IVM interacting proteins include endocytosis-related proteins. In the transcriptional level GO analysis, the results involved endosomal transport and macroautophagy-related genes, suggesting that IVM might be correlated to endocytosis or autophagy. Is it a possible tumor killing mechanism? Please comment.
6. In vivo, IVM is metabolized in the liver. In vitro, IVM acts directly on cultured cells. Is the mechanism of action the same?

7. In the CESTA method, the dose of IVM is 50microM, and the dose at the cellular level is 8-12microM. Will the proteins bound with the IVM be different?