

# Co-regulatory Network of Transcription Factors and miRNAs in Colorectal Cancer Pathogenesis

## Dear Editor

Accumulating evidence demonstrates the role of microRNAs (miRNAs) in a wide range of biological processes and pathological conditions such as oncogenesis.<sup>1</sup> Colorectal cancer (CRC) is a frequently encountered challenging type of cancer; therefore, exploring the trade-off between underlying biological units such as miRNAs and transcription factors (TFs) will probably lead to the identification of promising biomarkers involved in this malignancy. This study aims to investigate TFs and miRNAs regulatory relationships in CRC.

CRC curated genes were retrieved from OMIM (<https://www.ncbi.nlm.nih.gov/omim>), Cancer Genomics Consortium (CGC) (<https://www.cancer-genomics.org/>), and TCGA (<https://cancer-genome.nih.gov/>). CRC-specific unique miRNAs, in addition to their expression levels, were then obtained from miR2Disease (<https://omictools.com/mir2disease-tool>), PhenomiR2.0 (<http://mips.helmholtz-muenchen.de/phenomir/>), and HMDD2.0 (<http://www.cuilab.cn/hmdd>). Combinational interactions between the miRNAs, TFs, and genes were constructed by the TFmiR web server (<http://service.bioinformatik.uni-saarland.de/tfmir>) at a P value of 0.05 as the cutoff threshold. CRC-specific genes were functionally classified by using Reactome (<http://reactome.org/>) as underlying pathways, and their expression patterns were further sought using the Gemma server (<http://www.chibi.ubc.ca/Gemma/home.html>). Finally, the putative protein-protein hubs were sought among these genes by the EnrichR web server (<https://bio.tools/enrichr>) at a P value of 0.05.

Among 89, 63, and 211 CRC-specific unique miRNAs retrieved from miR2Disease, PhenomiR2.0, and HMDD2.0 databases, respectively, 89 common miRNAs and their expression levels were utilized for this analysis. Fifty-one CRC-specific genes obtained from OMIM, TCGA, and GCG databases were fed into the TFmiR web server in order to determine miRNA-TF and TF-gene links associated with CRC by default setting at a P value of less than 0.05. In the TF-gene links, *TP53*, *ELF3*, *EP300*, and *TCF7L7* TFs regulated the *CCND1*, *TGBFR2*, *DCC*, *MDM2*, *APC*, and *MSH2* genes. In the miRNA-TF regulatory network, 10 TFs including *TP53* and *EP300* were highlighted as the regulators of 17 miRNAs. However, no significant gene-miRNA regulatory interaction was found at the specified threshold. The expression patterns of the CRC-specific genes were finally checked in 26 transcriptomic experiments via the Gemma server. With the exception of the *APC*, *EDNRB*, *GPC6*, *MER3*, and *MLH1* genes, the remaining 46 genes exhibited a rather upregulation pattern in the experiments. The CRC-specific genes were mainly enriched in signaling pathways such as p53, Wnt, integrin, and TGF- $\beta$  in addition to angiogenesis and T-cell activation pathways. The SMAD family members such as SMAD3, SMAD2, SMAD4, and EP300 TF were found to be hubs within a protein-protein interaction network built by the EnrichR web server.

The results showed that 17 miRNAs including miR-145, miR-143, miR-15, miR-21, and miR-200c interacted with 9 TFs. Interestingly, most of the CRC-associated genes were enriched in angiogenesis, T-cell activation, and a number of signaling pathways such as Wnt, Integrin, TGF- $\beta$ , Ras, and p53, all of which have been previously characterized to be related to CRC development.<sup>2</sup> Among the TFs, *TP53*, *SMAD4*, and *AKT* were detected to interact with miR-145. In the TF-gene regulatory network, Cyclin D1 (*CCND1*) has been shown to adjust the occupation of EP300 TF at target DNA-binding sites in an independent kinase manner.<sup>2</sup> In the miRNA-TF regulatory network, EP300 was found to regulate miR-141 and miR-200c; moreover, *CCND1* regulated miR-20a. It is worthy of note that *CCND1* in this network was simultaneously regulated by *ELF3*, *EP300*, and *TCF7L2* while it seemingly acted as a mediator in the recruitment of *ELF3* on *TGFBR2*. The downregulation of *ELF3*, *EP300*, and *TCF7L2* causes the upregulation of *CCND1*. Hence, it is reasonable to argue that within this circuit, the upregulation of *CCND1* as a cell-cycle regulator in the p53 signaling pathway has a causal influence on cells toward tumorigenesis. In this network, *TP53* downregulates *APC* and *MDM2* and upregulates *DCC* and *MSH2*. A negligible upregulation (>2-fold change) in *TP53* probably indicates that it performs a role in tumor suppression by downregulating *APC* and *MDM2* involved in angiogenesis, as well as in the Wnt and p53 signaling pathways, which is contradicted by the role of *CCND1* in CRC progression, particularly by the ability of *CCND1* to inhibit apoptosis. Interestingly, the display of similar expression patterns by SMADs

and EP300 (log<sub>2</sub>[fold change]; P<0.05) among a wide range of experiments has been highlighted as hubs in the protein-protein interaction network. In the miRNA-TF interactions, *SMAD4* in tandem with *TP53* regulated miR-155, miR-145, and miR-143, which might be biologically relevant given the involvement of these units in setting a cascade of transcriptional regulatory events mediated by the p53 signaling pathway in CRC. Hao and colleagues<sup>3</sup> in their bioinformatics analysis not only showed which 5 deregulated miRNAs including miR-145 and 8 TFs were associated with CRC but also highlighted P13K/AKT as the main enriched pathway. Another network analysis indicated that miR-155-3p and miR-612 were involved in CRC metastasis through *TP53*-mediated signaling, which is in accordance with our study.<sup>4</sup> However, our analysis is challenged by the disadvantage of inevitable overestimation in computational approaches. Accordingly, the application of more stringent parameters in predicting regulatory links may confer more robust results. Our results demonstrated that the interactions between these genes and miRNAs and TFs predominantly occurred through angiogenesis and T-cell activation by perturbing the cell cycle. This can be considered a clue to CRC progression and be taken into account for future therapeutic targets in experimental endeavors.

Altogether, our study uncovered correlated alterations in gene expression that may be involved in CRC development and highlighted potential biomarker candidates for this disease.

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