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RAPID COMMUNICATION

The dysfunctional Wnt pathway down-regulates MLH1/SET expression and promotes microsatellite instability and immunotherapy response in colorectal cancer

The tumor heterogeneity results in different clinical characteristics of tumor patients, so precise therapy is a popular treatment strategy clinically. Microsatellite instability (MSI) is found in 10%–15% of colorectal cancer (CRC) patients and exhibits different clinical outcomes than microsatellite stable (MSS) patients. Surprisingly, MSI patients have a better prognosis than patients with stable microsatellites. MSI patients exhibit clinical features that are not responsive to chemotherapy, however, MSI patients showed excellent responses to immunotherapy.[1] Although the subtype classification of MSI is beneficial to the clinical treatment of CRC patients, the mechanism of MSI remains unclear.

Given that MSI is frequently studied in CRC, we integrated transcriptome data including 425 CRC patients to explore the underlying mechanism of MSI. Based on the pathway enrichment analysis, we found that the Wnt signaling pathway was enriched (Fig. 1A; Fig. S1A). Apparently, the canonical Wnt signaling pathway is inhibited in MSI patients (Fig. 1B; Fig. S1B). Besides, we also screened differentially expressed genes and found that Wnt pathway-related genes, especially *TCF7*, were significantly down-regulated (Fig. S1C–E).

The essence of MSI is the breakdown of the mismatch repair system. The results showed that mismatch repair enzyme gene *MLH1*, not *MSH2*, *MSH6*, or *PMS2*, is significantly down-regulated in MSI patients (Fig. S2A). This result suggests that *MLH1* may be regulated at the transcriptional level. Unlike previous reports,[2] there was no difference in the expression of DNA methyltransferases between MSS and MSI subgroups (Fig. S2B). To further verify the connection between the expression of *MLH1* and the Wnt signaling pathway, we analyzed the expression of *MLH1* under different small-molecule drug-stimulating conditions. The results showed that the expression of *MLH1* was significantly increased when the Wnt signaling pathway was activated. Conversely, when the Wnt signaling pathway was inhibited, the expression of *MLH1* was down-regulated (Fig. 1C, D). TCF7 acts as a transcription factor in the Wnt signaling pathway to regulate the expression of downstream target genes. We found that the *MLH1* promoter has the binding element for TCF7 (Fig. 1E). More importantly, we also found a positive correlation between *TCF7* and *MLH1* expression (Fig. S2C). SET has been identified as a biomarker of MSI in CRC.[3] Surprisingly, like *MLH1*, *SET* expression is regulated by the Wnt signaling pathway, and the binding element for TCF7 is present on the promoter (Fig. 1F–H; Fig. S3). These results suggest that *MLH1* and *SET* are down-regulated by Wnt/TCF7 in MSI patients.

When the Wnt pathway is activated, the interaction between TCF7 and β-catenin (encoded by *CTNNB1*) is required. However, we found that *CTNNB1* expression was elevated in MSI patients (Fig. S4A). In addition, we found that *RAC1* expression, which mediates the nuclear location of β-catenin, was reduced in MSI patients (Fig. S4B). In the CRC patients we collected, with the decrease of the expression of *MLH1*, the mismatch repair system gradually became abnormal, and the expression of *RAC1*, *TCF7*, and *SET* was decreased (Fig. 1I). These results suggest that Rac1 mediates the nuclear import of β-catenin and is involved in the regulation of target genes by the Wnt pathway. The piece of evidence supporting our conclusions is that the proportion of MSS patients in CRC was strikingly similar to the proportion of patients with nuclear β-catenin

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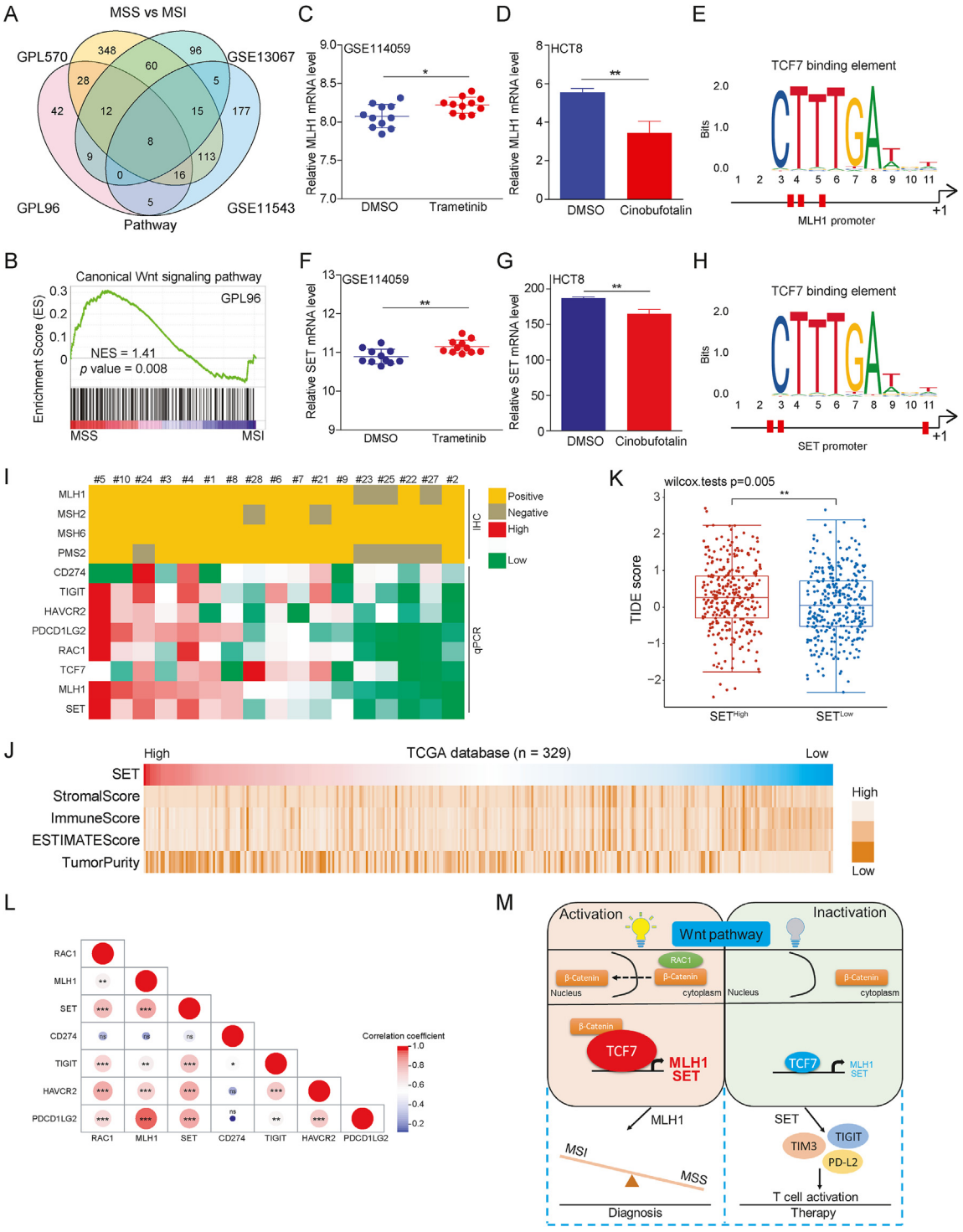

Figure 1 Abnormality of the Wnt pathway induces microsatellite instability (MSI) and regulates immune response in colorectal cancer. **(A)** The shared pathways were analyzed in microsatellite stable (MSS) and MSI colorectal cancer patients. **(B)** The canonical Wnt pathway was analyzed by GSEA in MSS and MSI colorectal cancer patients. **(C, D, F, G)** The expression of SET when the Wnt pathway was activated or inhibited. **(E, H)** The analysis of TCF7 binding elements was performed in SET promoter. **(I)** The expression of related genes was examined in colorectal cancer patients by qPCR. *Positive* means positive expression, and *Negative* means no expression. **(J)** The heat map showing immune infiltration score ranked according to SET expression in colorectal cancer patients from the TCGA database. **(K)** The distribution of immune response scores in SET^{high} and SET^{low} expressing colorectal cancer patients. **(L)** The expression correlations between target genes were analyzed in colorectal cancer patients. **(M)** Schematic representation of the regulatory role of the Wnt/β-catenin pathway in MSI. TIM3, protein coded by HAVCR2; PD-L2, protein coded by PDCD1LG2. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

expression. Moreover, the high level of nuclear β-catenin expression is closely related to the poor prognosis of CRC patients,[4] which is in line with the existing phenomenon that MSS patients have a worse prognosis than MSI patients. Second, among the shared differential pathways, DNA-binding transcriptional activity was also enriched (Fig. S1A), which also suggested changes in transcriptional-level regulatory processes in MSI. Third, in the results of PPI identification of core genes, in addition to TCF7, BCL9 is a protein component that binds to the target promoter after β-catenin enters the nucleus,[5] which also proves that the transcription activity of β-catenin/TCF7 is important for MSI. More importantly, we also found a significant positive correlation between the expression of *RAC1* and *MLH1*/*SET* in MSI patients (Fig. S4C, D). Futhermore, the genes identified (*RAC1*, *TCF7*, *MLH1*, and *SET*) were significantly reduced in MSI-H patients (Fig. S4E).

In this study, the down-regulation of *MLH1* induces MSI, but the role of *SET* in MSI has not been elucidated. In order to explore the function of SET in the process of MSI, we divided CRC patients into *SET* high- and low-expression groups and performed GSEA analysis. The results indicated that immune-related signaling pathways were activated in the *SET* low-expression group (Fig. S5A). Specifically, processes including monocyte migration and leukocyte-mediated immunity are activated when *SET* is low expressed (Fig. S5B). We also found that the immune cell infiltration increased with decreased *SET* expression in CRC patients (Fig. 1J). In addition, the proportion of different immune cells based on the high and low expression of *SET* is also different (Fig. S5C). Based on the TIDE algorithm, we found that low *SET* expression had a better response to immune checkpoint blockade (ICB) (Fig. 1K). In addition, we analyzed the effect of *SET* expression on the expression of known immune checkpoints and found that low-expressed *SET* significantly inhibited the expression of *CD274*, *HAVCR2*, *PDCD1LG2*, and *TIGIT* (Fig. S5D). Compared with MSS patients, *TIGIT*, *HAVCR2*, and *PDCD1LG2* showed a downward trend in MSI (Fig. 1I). In addition, we also found that *SET* and immune checkpoints (*TIGIT*, *HAVCR2*, and *PDCD1LG2*) have the significant positive correlation, but not *CD274* (Fig. 1L). This result strongly suggests that low expression of *SET* accompanied by low expression of immunosuppressive immune checkpoints in MSI patients increases the patient's response to immunotherapy. In conclusion, we found that inactivation of the canonical Wnt signaling pathway in MSI CRC patients resulted in decreased *MLH1* expression, defective mismatch repair system, and concomitant decreased *SET* expression, increased immune infiltration, and activated the immune system (Fig. 1M).

The classification of tumor subtypes is not only beneficial to the diagnosis of tumors but also to the treatment of tumors. By integrating and analyzing the sequencing data of MSI CRC patients, we found that Wnt abnormality is the driving force for the occurrence of MSI in the tumor patients. Decreased transcription factor and cofactor activity resulted in decreased expression of the mismatch repair enzyme *MLH1*, unable to repair DNA damage. Furthermore, the transcriptional target, *SET*, mediates immune checkpoints and immunotherapy in MSI patients, enhancing the response of MSI patients to immunotherapy. This study explored the molecular mechanism of MSI in tumors, hoping to provide some theoretical support for the clinical treatment of MSI tumors. However, due to a rigorous scientific attitude, these results require further experimental verification and corroboration of clinical results.

Conflict of interests

The authors have declared no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2023.03.026.

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