Inverse relation between FoxM1 and RASSF1A as a therapeutic target of Colon cancer

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ABSTRACT

Colorectal cancer (CRC) due to metastasis is the third leading cause of cancer death in the United States. Therefore, new research strategies and interventions are needed to identify key regulators for this metastatic colon cancer (mCRC). Metastatic CRC is the result of oncogene activation, such as Forkhead box M1 (FoxM1), and the loss of tumor suppressor genes (TSG), such as RASSF1A. Our objective is to test a regulatory link between oncogene inhibition and tumor suppressor gene upregulation in mCRC (T84, Colo 205, HCT-116), and the patient-derived organoid model (PDOD). The immunohistochemistry results demonstrated an inverse relationship in FoxM1 oncogene and RASSF1A TSG expression in different stages (I-IV) of colon cancer tissues. This inverse correlation was also observed in mCRC cell lines (T84, Colo 205) treated with Akt inhibitor (Wortmannin) or FoxM1 inhibitor (Thiostrepton). Additionally, the inhibition of FoxM1 expression in the PDOD model resulted in increased RASSF1A expression. Moreover, reduced levels of RASSF1A expression were found in normal cells (RWPE-1, HBEpc, MCF10A, EC) stimulated with exogenous $VEGF_{165}$. Down-regulation of FoxM1 coincides with an increase in the phosphorylation of YAP; conversely, downregulation of RASSF1A coincides with FoxM1 overexpression. Therefore, for the first time, these results demonstrate the suppression of angiogenesis signaling through any one of several steps inducing RASSF1A expression in metastatic colorectal cancer cells. These results also identify crosstalk between FoxM1 and RASSF1A, which could be used as a novel target to advance colon cancer treatment. However, the mechanism by which FoxM1 regulates expression and activity directly or through the YAP co-repressor in mCRC is not clear. Therefore, further investigation will be necessary to delineate its molecular interactions with FoxM1 and YAP to regulate RASSF1A expression in our lab based on the patient derived organoid model and in vivo model.

METHODS AND MATERIALS

Generation and Propagation of Patient-Derived Organoid Cell Cultures
Organoid cells were generated and cultured as previously described (Sato T., Gastroenterology 2011). The cultures were passaged when the aggregates reached a diameter of approximately 800 µm. Organoids were treated with 8 µM of thiostrepton (cyclic peptide antibiotic, FoxM1 inhibitor, Th) for 24 h and 48 h. Treated organoids and processed human tissues as standard protocol were subjected to immunoblotting.

Cell Culture
T84 and Colo 205 colon cancer cell lines were cultured with complete RPMI media and treated with AGP. To evaluate the relationship between suppression of angiogenic signal and RASSF1A expression, the colon cancer cells were treated with different concentrations of angiogenic inhibitor or neutralizing antibody. In details, T84 and Colo 205 cells were treated with neutralized VEGFR1 and VEGFR2 at the concentration of 0.25 µg/mL for 24 h (T84) and 48 h (Colo 205) whereas thiostrepton were used with different concentrations (0–8 µM) for 48 h and wortmannin were used (0–1 µM) for 24 h.

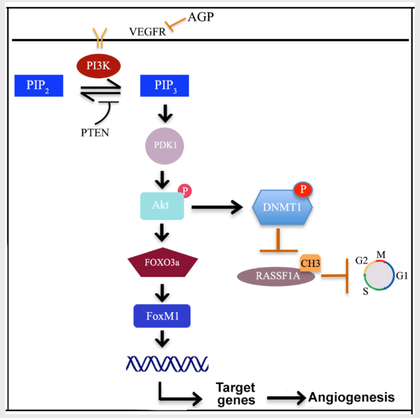
Immunohistochemistry
Unstained colon cancer tissue (stages I-IV) array with cancer and adjacent normal tissue as control. Two identical unstained tissues were subjected for immunohistochemistry as previously published protocol (Blanchard TG., Cancers 2019). .

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR)
Gene expression was evaluated Relative gene expression changes were calculated using the $2^{-\Delta\Delta CT}$ method, and expression normalization was accomplished using housekeeping gene GAPDH.

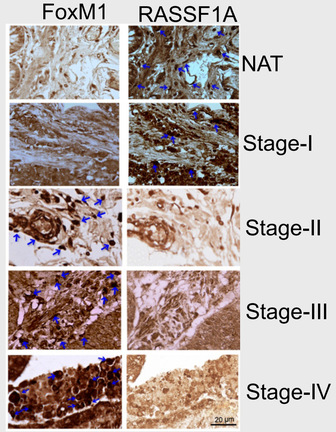
FoxM1 overexpression and depletion
As published protocol (Blanchard TG., Cancers 2019)

RESULTS

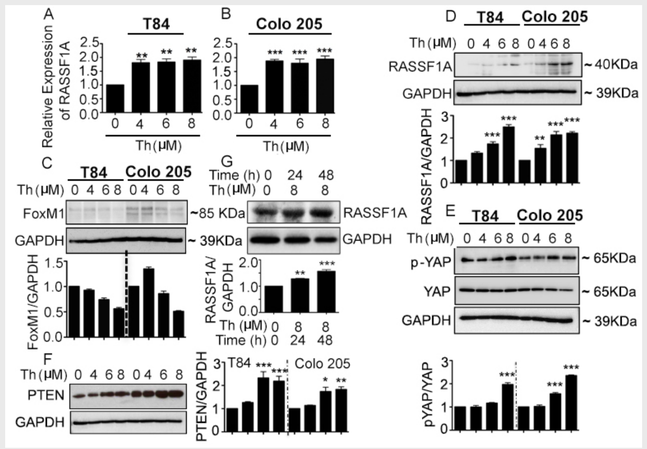
Crosstalk in between Angiogenic signal and RASSF1A



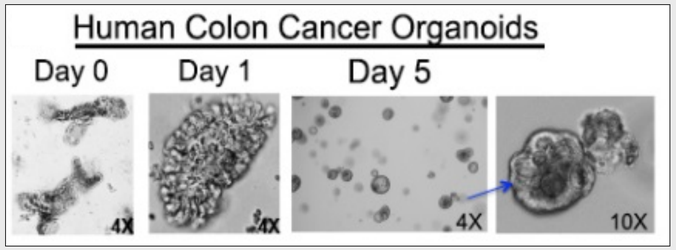
FoXM1 and RASSF1A expression



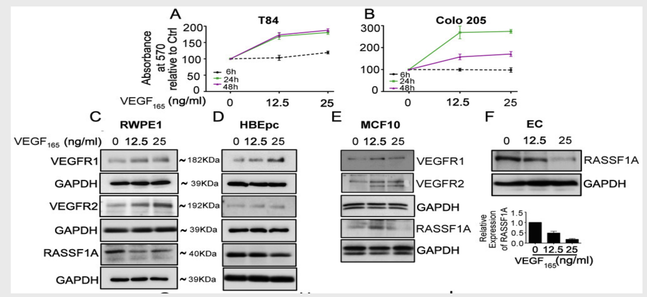
FoxM1 inhibitor induced RASSF1A expression



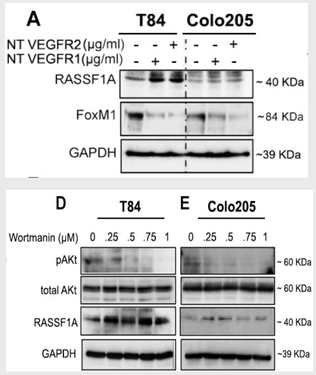
Human Colon Cancer Organoids



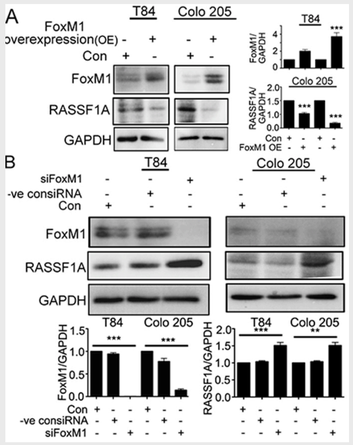
Exogenous VEGF suppressed RASSF1A



Impact of Angiogenic signal and RASSF1A expression



RASSF1A regulation is dependent on FoxM1



CONCLUSIONS

1. The underlying mechanism of mCRC involves the combination of FoxM1overexpression along with the suppression of RASSF1A.
2. First time our results demonstrate the suppression of angiogenesis signaling through any one of several steps induces RASSF1A expression in metastatic colorectal cancer cells.
3. identify crosstalk between FoxM1 and RASSF1A, which could be used as a novel target to advance colon cancer treatment

Acknowledgements

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