International Society of Gastrointestinal Oncology
2011 Gastrointestinal Oncology Conference
September 15–17, 2011
ABSTRACTS

Advanced Colorectal Cancer

abstr 1111

Identification of MicroRNAs Differentially Expressed in Normal Colon and Colon Cancer Stem Cells

Shigeo Hisamori, Piero Dalerba, Yohei Shimono, Michael E Rothenberg and Michael F Clarke

Stanford Institute for Stem Cell Biology and Regenerative Medicine, Stanford University, Stanford, CA, USA

Background. A growing body of evidence indicates that human colon cancer contains a "colon cancer stem cell" (CoCSC) population with properties reminiscent of normal colon stem cells (NCSCs). Only these cells have an ability to self-renew and can give rise to the original tumor in mouse transplantation experiments. MicroRNAs (miRNAs) are endogenous small noncoding RNAs, 18-25 nucleotides in length, which suppress gene translation by binding to specific seed sequences in the 3'untranslated region (3'UTR) of target mRNAs. We aimed to find miRNAs whose expression is modulated during differentiation process in the normal colon epithelium and investigate their role in the regulation of self-renewal and differentiation abilities in both normal colon and colon cancer tissues.

Methods. We isolated similar numbers of "bottom of the crypt" immature progenitor cells ($EpCAM^{high}$, $CD44^{+}$) and "top of the crypt" mature cells ($EpCAM^{high}$, $CD44^{-}$, $CD66a^{+}$) from both normal and colon cancer primary samples by fluorescence-activated cell sorting (FACS), and analyzed their miRNA expression profile using real-time PCR. We evaluated the direct effect of these miRNAs to regulate the 3'UTR of candidate target genes via luciferase reporter assays, and tested their abilities to downregulate the corresponding endogenous proteins by Western Blotting. To determine how these miRNAs might affect the tumorigenic potential of colon cancer cells, we infected CoCSCs from human colon cancer xenograft with lentivirus encording for miRNAs that were shown to be downregulated in CoCSCs, and investigated the changes of their organoid-formation ability *in vitro* and tumorigenicity *in vivo*.

Results. We found several miRNAs that were differentially expressed between top and bottom of the crypt of human normal colon tissues. Among them, miR-203 and miR-200c were downregulated in human CoCSCs and in both human and murine NCSCs. We observed that the miR-203 precursor suppressed the luciferase activity of the reporter vector encording the wild-type 3'UTR of the *TCF7L2* mRNA, which encodes the TCF4 transcription factor, an important downstream effector of the canonical Wnt β-catenin pathway. Mutation of the miR-203 seed region within the *TCF7L2* 3'UTR abrogated the repressive ability of miR-203. Both miR-203 and miR-200c precursors suppressed the luciferase activity of the vector encording the wild-type 3'UTR of *BMI1*, and mutation of the miR-203 or miR-200c seed region within the *BMI1* 3'UTR abrogated the repressive ability of miR-203 or miR-200c, respectively. Western blotting showed that TCF4 protein expression was decreased in colon cancer cell lines transfected by miR-203, and that BMI1 protein expression was decreased in cell lines transfected by miR-203 or miR200c. Forced expression of miR-203 and/or miR-200c inhibited the clonal expansion of the SW620 colon cancer cell line and the organoid-formation ability of human xenograft CoCSCs *in vitro*. Moreover, miR-203 suppressed the *in vivo* tumorigenic capacity of human CoCSCs when injected subcutaneously in NSG mice.

Conclusions. MiR-203 and miR-200c are downregulated both in NCSCs and CoCSCs as compared to their more mature progeny, and suppress the expression of TCF4 and BMI1. These findings suggest that normal stem/progenitor cells and CoCSCs share a similar molecular machinery to regulate self renewal and differentiation processes.