MicroRNA miR-29a inhibits colon cancer progression by downregulating B7-H3 expression: potential molecular targets for colon cancer therapy

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Abstract

Background

MiR-29a belongs to one of the subtypes of miRNAs known as non-coding single-stranded RNAs, and is preferentially expressed in normal tissues. B7-H3, a member of the B7/CD28 immunoglobulin superfamily, was shown to be overexpressed in several solid malignant tumors, including colon cancer. In addition, it is associated with tumor progression and poor prognosis.

Methods

We used immunohistochemical and western blotting to assess B7-H3 protein expression levels in colon cancer and adjacent normal tissues, and then compared their relationships with clinicopathological factors. Quantitative real time reverse transcription PCR was used to assess B7-H3 and miRNA-29a mRNA expression levels, and then their relationship and clinical significance were evaluated. In addition, colon cancer Caco-2 cells, which constitutively overexpress B7-H3, were transfected with lentivirus particles for miR-29a upregulation. Invasion and migration assays were carried out in vitro along with the establishment of a subcutaneous xenograft model in vivo to determine the role of miRNA-29a in colon cancer progression.

Results

The B7-H3 protein showed elevated expression in colon carcinoma, and was relevant to TNM staging, lymph node metastasis and reduced survival. Meanwhile, miR-29a was preferentially expressed in normal colon tissues while B7-H3 transcript levels had no marked differences between tumor and normal tissue specimens. In vitro, miR-29a upregulation resulted in reduced B7-H3 expression. Furthermore, miR-29a upregulation reduced the invasive and migratory abilities of colon carcinoma cells. In animal models, upregulation of miR-29a slowed down the growth of subcutaneous xenotransplanted tumors, and resulted in prolonged survival time.

Conclusion

MiR-29a downregulates B7-H3 expression and accordingly inhibits colon cancer progression, invasion and migration, indicating miR-29a and B7-H3 might represent novel molecular targets for advanced immunotherapy in colon cancer.

Full Text

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Figures

Figure 1

Immunohistochemical staining of colon cancer and adjacent normal tissue specimens (magnification, 200×). Negative (A) and positive (B) expression of B7-H3 in colon cancer tissue samples. Negative (C) and positive (D) expression of B7-H3 in adjacent normal tissues.

Figure 2

Kaplan-Meier curves showing survival based on B7-H3 expression in colon cancer tissues. Compared with patients not expressing B7-H3, the B7-H3 positive expression group showed shorter survival time ($P=0.022$).

Figure 3

B7-H3 mRNA and miR-29a expression levels in colon cancer- and adjacent normal colon tissues determined by qRT-PCR, and B7-H3 protein expression levels assessed by Western blotting. (A) The average value of mRNA expression levels in adjacent normal colon tissues was set as 1, to derive relative amounts in colon cancer tissues. There was no significant differences between colon cancer- and adjacent normal colon tissues in B7-H3 mRNA expression levels ($P>0.05$). (B) MiR-29a expression levels in colon cancer tissues were obviously higher than those of colon cancer tissues. (C) B7-H3 protein levels in colon cancer tissues were remarkably higher than those of adjacent normal colon tissues. * $P<0.05$, ** $P<0.01$, *** $P<0.001$.

Figure 4

MiR-29a downregulates B7-H3. (A) MiR-29a expression levels were determined by qRT-PCR after Caco-2 cell transfection with lentiviral particles harboring miR-29a, anti-miR-29a and negative control, respectively. The average value of miR-29a expression levels in the LV-NC group was set as 1, to derive the relative expression levels in the other groups. The miR-29a expression levels in the LV-miR-29a group were higher compared with those of the LV-NC group; the anti-miR-29a group showed the opposite trend. (B) B7-H3 expression levels were determined by flow cytometry, and were lower in the LV-miR-29a group compared with the LV-NC group, but higher in the anti-miR-29a group. A non-specific MoAb served as the staining control. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 5

Effects of miR-29a on Caco-2 colon cell invasion and migration. (A, B) The wound healing assay showed that wound healing was reduced in LV-miR-29a transfected Caco-2 cells but increased in the LV-anti-miR-29a group, compared with LV-NC transfected Caco-2 cells. (C, D) The transwell invasion assay showed reduced invasive ability in LV-miR-29a transfected Caco-2 cells but increased ability in the LV-anti-miR-29a group, compared with LV-NC transfected Caco-2 cells. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure 6

Effects of miR-29a on subcutaneous xenotransplanted tumors and mouse survival. (A) Tumor sizes were larger in the LV-anti-miR-29a group but reduced in the LV-miR-29a group, compared with the LV-NC group. (B) Survival time was longer in the LV-miR-29a group but shorter in the LV-anti-miR-29a group, compared with the LV-NC group. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.