The role of miR-378 in sarcomatoid renal cell carcinoma

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Abstract

Sarcomatoid renal cell carcinoma (sRCC) is considered as aggressive, highly metastatic and drug resistance RCC subtype. According to our previous studies, elevating the expression of miR-378 could result in apoptosis. Therefore, we hypothesized that modulating miR-378 expression levels in RCC cells may suppress the tumour cell proliferation and activate the intrinsic pathway of apoptosis. After increased the expression levels of miR-378 in tumor cells, both RCC subtypes significantly induced all cells to apoptosis. Most notably sRCC represented significant down-regulation of mRNA and protein expression of POLR2A and RUNX2 when compared to clear cell renal cell carcinoma (ccRCC) cells or control cells. The miR-378 might act as an important role of tumour suppressor, and the potential effects of the POLR2A and RUNX2 genes on the prognosis of sRCC should be considered.

Material and Method

We interpreted the correlation of miR-378 and two candidate genes (POLR2A and RUNX2) in RCCs by using RT-qPCR, western blotting and flow cytometry in current study. The study involved five cell lines (included three ccRCCs, one sRCC and one control cells). Real-time polymerase chain reaction and western blot analysis were performed in all cell lines to evaluate the expression of mRNA and protein in POLR2A and RUNX2 before and after transfection of miR-378. Finally, apoptosis was analyzed by flow cytometry.

Result

Figure 1. Using q-PCR to analyze mRNA expression levels of POLR2A in RCC cell lines

Figure 2. Using q-PCR to analyze mRNA expression levels of RUNX2 in RCC cell lines

Figure 3. Differences of protein expression of POLR2A between pre-improve and post-improve miR-378.

(A) Using western blotting to analyze the protein expression level of POLR2A in each RCC cell line. (B) Using image J to standardize the expression level of GAPDH. (pre-improved : Control group · post-improved : Added miR-378)

Figure 4. Differences of protein expression of RUNX2 between pre-improve and post-improve miR-378.

(A) Using western blotting to analyze the protein expression level of RUNX2 in each RCC cell line. (B) Using image J to standardize the expression level of GAPDH. (pre-improved : Control group · post-improved : Added miR-378)

Figure 5. Using flow cytometry to analyze the proportion of apoptosis in each RCC cell line

(A) Control group: pre-improve miR-378 group. (B) Test group (added miR-378): post-improve miR-378. HK-2 (normal renal cell) · 786-O · A498 · Caki-1 (ccRCC) · RCC52 (sRCC). Statistics are done via Bonferroni post-tests in two-way ANOVA.

Conclusion

1. Our data indicated that miR-378 might be a biomarker of RCCs, especially sRCC.
2. miR-378 can inhibits the mRNA and protein expression of POLR2A and RUNX2 which is overexpress in sRCC, which leads to apoptosis of RCC cell lines.
3. Interestingly, we surmise that the different site mutations in cells will lead to inconsistent expression of mRNA and protein in ccRCC cell lines.
4. It is obvious that a miR-378 is able to regulate cellular genes.

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