A new prognostic marker- Interferon regulatory factor 6 in renal cell carcinoma

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Background

According to our previous methylated-CpG island recovery assay (MIRA) and RNA expression array findings, the results indicated methylated status of Interferon regulatory factor 6 (IRF6) could be observed in most of renal cell carcinoma (RCC) cases (40.6%), and presented a negative correlation with gene expression, clear cell type of RCCs especially. As known that IRF6 is one of members of IRF family. It has been described might play an important role in epidermal cells, and correlated with cell proliferation and differentiation. The regulation of feedback loop between IRF6 and ΔNp63, which is known could control the mechanism of keratinocytes differentiation and palate closure, has also been noted. In addition, the tumor suppressor activity of IRF6 had been also demonstrated in squamous carcinoma associated with inhibition of tumor cell invasion and migration. The aim of this study is to clarify the clinical significance and role of IRF6 in RCC. We hypothesized low expression of IRF6 via DNA methylation might lead to irregular differentiation of RCC cells, and then further trigger cells proliferated out of control.

Materials and methods

In total, 105 pairs of clinical RCCs patients and eight RCC cell lines, as well as Human Embryonic Kidney 293 cell used as control have involved in current study. Firstly, we performed real-time PCR on all cases. Secondarily, 5-aza-2′-deoxycytidine was treated on all type of RCC cell lines; it is a way of demethylated DNA. Western blot detections were then to confirm whether the expression level of IRF6 restored in RCC cells. The IRF6 gene expressed levels differ from normal and RCC tissues were applied by the paired- T test, and shown by ΔCT.

Figure 1. Relative gene expression level of normal and RCC tissues as shown by -ΔCT.

Results

Generally, the variant and lower level gene expression of the IRF6 could be observed from all different type of RCC cell lines, except RCC98 and RCC100 cells. After treated with 5-aza-2′-deoxycytidine on RCC cell lines, the obvious expression of IRF6 was restored in all clear cell RCC cell lines. The mean – ΔCT of normal tissue was -8.0, and RCC tissue was -11.5, significantly IRF6 expressed levels differ from normal and tumor tissues could be noted (p=0.013).

Figure 2. The gene expression of IRF6 in different types of RCC cell lines (chromophobe type RCC98, papamoid type RCC52, clear cell type Caki-1, HH332, H0KN-9, RCC100, papillary type HB050 and adenocarcinoma 786-O).

(A) Quantitative Real-time PCR. (B) Western blot analysis.

Figure 3. All cell lines were treated with 10μM 5-aza-2′-deoxycytidine six days to restored the expression of IRF6.

(A) Quantitative Real-time PCR results of IRF6 mRNA levels that treated with 5-aza-2′-deoxycytidine in RCC cell lines. (B) Western blot analysis results show that demethylation can affect the expression of protein.

Conclusion

1. The IRF6 gene was usually expressed lower level in RCC due to methylation.
2. The expression of IRF6 could be easily restored by de-methylated in RCC cells, and it might further inhibit cell proliferation in the tumoral progression, and might consider as a prognostic marker to predict patients’ outcomes in RCC.
3. The further cell viability and correlation with the clinical information should be further analyzed in the future.

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