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Original Articles

Interferon regulatory factor 8 functions as a tumor suppressor in renal cell carcinoma and its promoter methylation is associated with patient poor prognosis

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ABSTRACT

Interferon regulatory factor 8 (*IRF8*), as a central element of IFN-γ-signaling, plays a critical role in tumor suppression. However, its expression and underlying molecular mechanism remain elusive in renal cell carcinoma (RCC). Here, we examined *IRF8* expression and methylation in RCC cell lines and primary tumors, and further assessed its tumor suppressive functions. We found that *IRF8* was widely expressed in human normal tissues including kidney, but frequently downregulated by promoter methylation in RCC cell lines. *IRF8* methylation was detected in 25% of primary tumors, but not in adjacent non-malignant renal tissues, and associated with higher tumor nuclear grade of RCC. Ectopic expression of *IRF8* inhibited colony formation and migration abilities of RCC cells, through inducing cell cycle G2/M arrest and apoptosis. IFN-γ could induce *IRF8* expression in RCC cells, together with increased cleaved-PARP. We further found that *IRF8* inhibited expression of oncogenes *YAP1* and *Survivin*, as well as upregulated expression of tumor suppressor genes *CASP1, p21* and *PTEN*. Collectively, our data demonstrate that *IRF8* as a functional tumor suppressor is frequently methylated in RCC, and *IRF8*-mediated interferon signaling is involved in RCC pathogenesis.

Introduction

Renal cell carcinoma (RCC) is the highest mortality rate of the genitourinary cancers [1], with a steadily increased incidence, which accounts for ~90% of kidney cancer cases in adults. Deciphering molecular mechanisms underlying RCC tumorigenesis is critical for developing better biomarkers for its early diagnosis and prognosis. Emerging evidence has demonstrated that accumulated genetic and epigenetic alterations can lead to tumor initiation and progression including RCC [2–4]. Of note, mutation is not a frequent event in RCC [5]. A large scale mutation analysis of RCC showed that few genes are mutated in >15% of tumors, except *von Hippel–Lindau* (*VHL*) (52%) and *PBRM1* (41%) (http://www.sanger.ac.uk/genetics/CGP/cosmic/) [6,7], suggesting alternative epigenetic abnormities involving in renal tumorigenesis.

Epigenetic alterations, including promoter CpG methylation and histone modification, mediate oncogene activation and tumor suppressor gene (TSG) inactivation, thus contribute to tumorigenesis. Multiple TSGs with aberrant promoter methylation have been found in RCC, such as *VHL, Ras association family 1A (RASSF1A), secreted frizzled-related protein 1* (*SFRP1*), *cadherin 1 (CDH1)* and *p16* [8–12]. We have identified some TSGs silenced by promoter methylation in RCC, including *RASAL1, deleted in lung and esophageal cancer (DLEC1)* and *deleted in liver cancer 1 (DLC1)*, and *DLEC1* could be a potential prognostic biomarker for RCC [13–15]. These findings not only uncover molecular heterogeneity of RCC, but provide an attractive strategy for RCC biomarker discovery. As relatively low frequency of TSG methylation found in RCC (~10–30%), identifying more novel TSGs inactivated by promoter methylation in RCC is needed.

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Interferon regulatory factor 8 (*IRF8*), also known as interferon consensus sequence-binding protein (*ICSBP*), is a transcription factor of the interferon (IFN) regulatory factor (IRF) family [16], which as a central element of IFN-γ-signaling modulates multiple physiological processes [17–21]. Previous studies of *IRF8* were mainly concentrated on cells of myeloid and lymphoid lineages. Recently, its expression and function in other types of human solid tumors have been demonstrated by us and others. Our previous studies showed that *IRF8* was frequently silenced by promoter methylation in nasopharyngeal, esophageal, lung, colon and breast carcinomas, thus as a TSG [22]. However, its expression and biological function in RCC pathogenesis remain unclear.

In this study, we examined *IRF8* expression and methylation in RCC cell lines and primary tumors, analyzed the relationship between its methylation and clinicopathological features in RCC patients. We also investigated its tumor suppressive function in RCC cells, including inhibition of clonogenicity and migration abilities, cell cycle arrest and apoptosis, as well as further identified its target genes.

Table 1
Association of clinicopathological features with *IRF8* methylation status in renal cancer.

Clinicopathologic features		Number ($N = 44$)	*IRF8* methylation status		p value
			Methylated	Unmethylated	
Gender	Male	33	8	25	*0.565*
	Female	11	3	8	
Side	Rt	21	8	13	0.058
	Lt	23	3	20	
TNM classification	pT1a	24	6	18	0.835
	pT1b	9	2	7	
	pT2	6	1	5	
	pT3	5	2	3	
Nuclear grade	G1	9	1	8	0.001
	G2	22	3	21	
	G3	10	7	3	

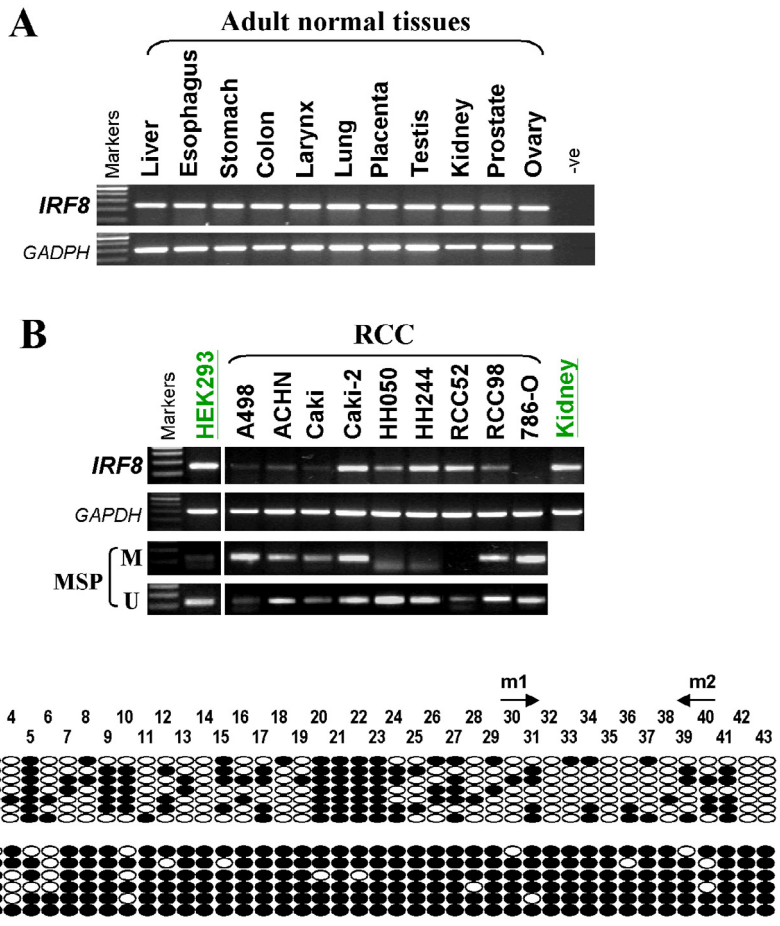

Fig. 1. *IRF8* silencing by promoter methylation in RCC cell lines. (A, B) Detection of *IRF8* expression in human adult normal tissues and a panel of RCC cell lines by semi-quantitative RT-PCR. M, methylated; U, unmethylated. (C) Bisulfite genomic sequencing (BGS) analysis of the *IRF8* promoter in HEK293 cell line and RCC A498 cell line. Circles, CpG sites analyzed; row of circles, an individual promoter allele that was cloned, randomly selected, and sequenced; filled circle, methylated CpG site; open circle, unmethylated CpG site.