Predictive values of Notch signalling in renal carcinoma

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Submitted: 5 July 2016
Accepted: 30 October 2016

Arch Med Sci 2017; 13, 6: 1249–1254
DOI: https://doi.org/10.5114/aoms.2017.65649
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Abstract

Introduction: Notch signalling, an evolutionarily conserved mechanism of cellular differentiation and tissue remodelling, is frequently deregulated in several human malignancies, including renal cell carcinoma (RCC). However, the prognostic value of individual Notch pathway members in RC subtypes remains indefinable. The present study investigates whether the differential expression of Notch members has a contrary effect on disease-free survival (DFS) in clear cell renal cell carcinoma (KIRC), papillary cell renal cell carcinoma (KIRP) and chromophobe renal cell carcinoma (KICH) patients.
Material and methods: The predictive value of 19 Notch members was evaluated in KICH, KIRC and KIRP patient cohorts from The Cancer Genome Atlas (TCGA). Results in the form of Kaplan-Meier survival plots with the *p*-value calculated (log-rank test, $p < 0.05$) enabled the patients to be split into favourable/unfavourable prognosis groups regarding expression of Notch members.
Results: More specifically, lowered expression of ADAM17 correlated with good prognosis in KICH, KIRC and KIRP (HR = 7.79, $p = 0.03$; HR = 3.98, $p = 0.051$; HR = 11.24, $p < 0.001$, respectively). Additionally, HES4 differentiated KICH and KIRC, as its higher expression correlated with good prognosis in KICH and favourable lowered expression in KIRC (HR = 0.11, $p = 0.015$; HR = 2.42, $p < 0.001$, respectively).
Conclusions: Our analysis could be valuable for better understanding of the molecular mechanism of renal carcinoma. The expression of Notch pathway members could be a useful biomarker for predicting favourable/unfavourable prognosis in patients with RCC.

Key words: kidney neoplasms, disease-free survival, prognosis, biomarkers.

Introduction

The kidney is a specific organ comprising various types of cells. Therefore, kidney cancer may occur in a number of different and specific types that can be characterized by different histologies, different clinical courses and differing responses to a number of varied therapies. To date, the majority of renal cell carcinomas with specified subtypes are the clear cell type (KIRC), followed by papillary (KIRP) and chromophobe (KICH) tumours [1, 2].

The Notch pathway is an evolutionarily conserved signalling mechanism involved in the regulation of proliferation, differentiation, vascular remodelling and angiogenesis in embryonic and adult tissues [3]. The canonical Notch pathway is activated by the interaction of DSL ligands (DLL1, DLL3, DLL4, JAG1 and JAG2) and Notch receptors (NOTCH1-NOTCH4) leading to two sequential proteolytic cleavages of

the receptors. The first cleavage involves ADAM/TACE metalloprotease, and the remaining portion of Notch is subsequently cleaved by the γ-secretase complex (composed of PSEN1, PSEN2, PEN2, APH1, and nicastrin). A second cleavage is then followed by the release of the Notch intracellular domain (NICD) to the nucleus, where it forms a complex with the DNA binding protein RPBJ and MAML family transcriptional coactivators. The latter induces the expression of Notch downstream effectors, such as transcription factors (TFs), i.e. *HES1* and *HEY1* [4, 5].

Notch plays a key role in kidney development by establishing a proximal tubular epithelial cell fate and cell type specification in the renal collecting system [6]. Moreover, it has been proven that aberrant Notch signalling may result in tumourigenesis. For example, a study by Aparicio *et al*. revealed higher *NOTCH1* expression in KICH tissues [7]. In turn, reduced Notch signalling was found in KIRP, as demonstrated by gene expression analysis indicating that the Notch downstream effector (*HEY1*) was reduced [8]. Nevertheless, as little is known about the prognostic value of Notch members and their influence on disease recurrence, especially in the kidneys, the aim of the present study was to investigate the potential effect of Notch differential expression on disease-free survival (DFS) in KICH, KIRC and KIRP.

Material and methods

We obtained the mRNASeq data of 973 cancer samples (RNA-Seq, level 3 RNASeqV2, RSEM normalized) (data status of Jan 28, 2016) and matched clinical data of the renal carcinoma KIPAN cohort (KICH + KIRC + KIRP) from The Cancer Genome Atlas (TCGA), downloaded from http://gdac.broadinstitute.org/. All TCGA samples have been collected, RNA isolated and sequenced according to Institutional Review Board approval of the protocols; see the project website at http://cancergenome.nih.gov for more details [9–11].

Patients with missing clinical/expression values were excluded from further analyses. Finally, a total of 888 samples were qualified: 66 KICH, 533 KIRC and 289 KIRP patients. The clinical characteristics of the patient cohort are presented in Table I.

Previously prepared KICH, KIRC and KIRP data were used to determine the relevance of the expression of 19 Notch signalling pathway members to disease-free survival. The analysis was based on optimal cutoff point determination using the freely available Cutoff Finder web application (http://molpath.charite.de/cutoff/). The clinical characteristics defining DFS were as follows: "patient.days_to_last_followup" for survival time and "patient.follow_ups.follow_up.person_neoplasm_cancer_status" for outcome and event.

Statistical analysis

The significance of the correlation with the survival variable was chosen for optimizing the cutoff point, defined as the point with the most significant split. Additionally, hazard ratios (HRs) including 95% confidence intervals (CI) were calculated [12]. Results in the form of Kaplan-Meier survival plots with the *p*-value calculated (log-rank test, $p < 0.05$) enabled us to split patients into favourable/unfavourable prognosis groups regarding expression of Notch members.

Results

The present study analyses the influence of differential expression of Notch members on DFS in KICH, KIRC and KIRP patients. Table II presents the cutoff points and numbers of patients assigned to groups of low and high expression of Notch members. Contrasting DFS Notch profiles were found across kidney carcinomas. Firstly, lowered expression of *ADAM17* correlated with good prognosis in KICH, KIRC and KIRP (HR = 7.79, $p = 0.03$; HR = 3.98, $p = 0.051$; HR = 11.24, $p < 0.001$, respectively) (Figure 1). While lowered expression of *NUMB* was favourable in KICH and KIRP (HR = 6.7, $p = 0.016$; HR = 4.09, $p < 0.001$, respectively), higher expression was favourable in KIRC (HR = 0.21, $p = 0.017$) (Figure 1). In contrast, while high *PSEN2* expression correlated with good prognosis in KICH and KIRP (HR = 0.2, $p = 0.048$; HR < 0.001, $p = 0.023$, respectively), its lowered expression was favourable in KICH (HR = 2.81, $p < 0.001$) (Figure 1). Lowered expression of the *DLL4*, *HEY1*, *JAG2*, *NOTCH1*, *NOTCH3* and *NOTCH4* genes was favourable in KIRC and KIRP, while higher expression of *APH1B* was favourable in KIRC and KIRP (HR = 0.53, $p = 0.028$; HR = 0.15, $p < 0.001$, respectively). *HES4* was found to differentiate between KICH and KIRC, as its higher expression correlated with good prognosis in KICH while its lowered expression was favourable in KIRC (HR = 0.11, $p = 0.015$; HR = 2.42, $p < 0.001$, respectively). Finally, *ADAM10*, *HES1* and *PSEN1* were significant for DFS in KIRC, *HES5* and *JAG1* in KIRP and *NOTCH2* in KICH (Table II).

Discussion

Renal cell carcinoma (RCC), the most common tumour of the adult kidney, displays heterogeneous histologic characteristics, with the majority of cases being KIRC (70–75%), and the remainder comprising KIRP (about 10 % of cases) and KICH (5%) [13]. Despite recent progress, new biomarkers and therapeutic targets of renal carcinoma need to be established to overcome the resistance of kidney cancer to various kinds of therapy. The aim of the present study was to evaluate the prog-

Table I. Clinical characteristics of KIRC, KIRP and KICH cohort patients

Parameter	Total	Males	Females
KIRC:			
Quantity	533	345	188
Median age (range)	61 (26–90)	59 (26–90)	63 (29–90)
Stage:			
I	267	161	106
II	57	43	14
III	123	80	43
IV	84	59	25
NA	–	–	–
KIRP:			
Quantity	289	213	76
Median age (range)	62 (37–88)	62 (40–85)	62 (37–88)
Stage:			
I	177	132	45
II	25	17	8
III	53	38	15
IV	17	12	5
NA	17	14	3
KICH:			
Quantity	66	39	27
Median age (range)	50 (17–86)	53.5 (26–78)	46 (17–86)
Stage:			
I	22	11	10
II	25	13	12
III	14	10	4
IV	6	5	1
NA	–	–	–

NA – not available.

nostic effect of the expression of Notch pathway members on DFS in renal carcinoma. Initially, although the effect of 19 genes involved in the Notch pathway were studied, only three of them were found to be significantly associated with a tumour relapse prognosis in all three subtypes (Table II).

ADAM17 has been found to play a causative role in the development and progression of many cancers and may participate in the tumorigenesis of renal cancer. It has been reported that *ADAM17* mRNA was highly expressed in renal carcinoma [14] and its level correlated positively with tumour stage [15]. Furthermore, it has been identified that *ADAM17* is frequently expressed in metastatic KIRC and in localized KIRC, and importantly, high expression of *ADAM17* was associated with reduced progression-free survival in patients with KIRC [16]. Our present findings indicate that lowered expression of *ADAM17* was correlated with favourable DFS prognosis in all three subtypes of renal carcinoma.

NUMB is an evolutionarily conserved protein that controls multiple development processes such as asymmetric cell division, cell fate choice, cellular adhesion and cell migration. Studies have shown that NUMB-dependent events play an important role in various tumours [17]. Sima *et al.* demonstrated that *NUMB* has suppressive potential on the KIRC cell lines 786-0, Caki-1 and Caki-2, and that NUMB protein expression was decreased in the KIRC cell compared with control cells ($p < 0.001$). In addition, ectopic *NUMB* expression inhibited proliferation, migration and invasion, and this effect may be caused by the downregulation of cyclin D1 or *MMP-9* [18]. As expected, a favour-

Table II. Statistics for DFS analysis

Gene	Cut-off	Number of patients in group		HR	*P*-value
		Low expression*	High expression*		
KICH:					
ADAM17	290.9	40	26	7.79	0.03
HES4	14.17	21	45	0.11	0.015
NOTCH2	339.9	33	33	> 100	0.011
NUMB	524.1	19	47	6.7	0.016
PSEN2	2729	52	14	0.2	0.048
KIRC:					
ADAM10	4152	513	20	5.54	0.0017
ADAM17	963.5	499	34	3.98	0.0051
APH1B	460.7	219	314	0.53	0.028
DLL4	6186	521	12	6.36	< 0.001
HES1	3005	512	21	2.2	< 0.001
HES4	230.7	458	75	2.42	0.0064
HEY1	1072	517	16	4.83	0.001
JAG2	2251	517	16	3.64	0.022
NOTCH1	800.4	63	470	6.05	0.043
NOTCH3	6697	276	257	1.77	0.051
NOTCH4	7208	518	15	4.38	0.0074
NUMB	2979	461	72	0.21	0.017
PSEN1	1534	215	318	0.44	0.0051
PSEN2	407.2	261	272	2.81	< 0.001
KIRP:					
ADAM17	875.4	271	18	11.24	< 0.001
APH1B	131.1	16	273	0.15	< 0.001
DLL4	518	273	16	5.45	0.0026
HES5	3.4	258	31	6.78	< 0.001
HEY1	46.53	165	124	4.17	0.0017
JAG1	1049	65	224	> 100	0.01
JAG2	642.9	272	14	3.27	0.047
NOTCH1	1504	269	20	4.05	0.0072
NOTCH3	1237	231	59	3.78	0.0016
NOTCH4	599.4	265	24	3.98	0.0026
NUMB	3354	278	11	4.09	0.0079
PSEN2	542.1	224	65	< 0.001	0.023

**We defined "low expression" as the expression values below and "high expression" as the expression values above the determined cut-off.*

able DFS prognosis was found to be associated with elevated expression of *NUMB* in KIRC, which would confirm its suppressive character. Surprisingly, high *NUMB* expression turned out to be significantly correlated with poorer patient prognosis in KICH and KIRP. Together, these findings may indicate that *NUMB* plays a binary role in tumorigenesis: as a suppressor in KIRC and an oncogene in KICH and KIRP.

Presenilin 2 (PSEN2) is a member of the γ-secretase complex, a multi-subunit protease complex involved in intramembrane proteolysis of NOTCH extracellular truncation (NEXT) [5]. Mutations in the PSEN2 protein have been widely reported in

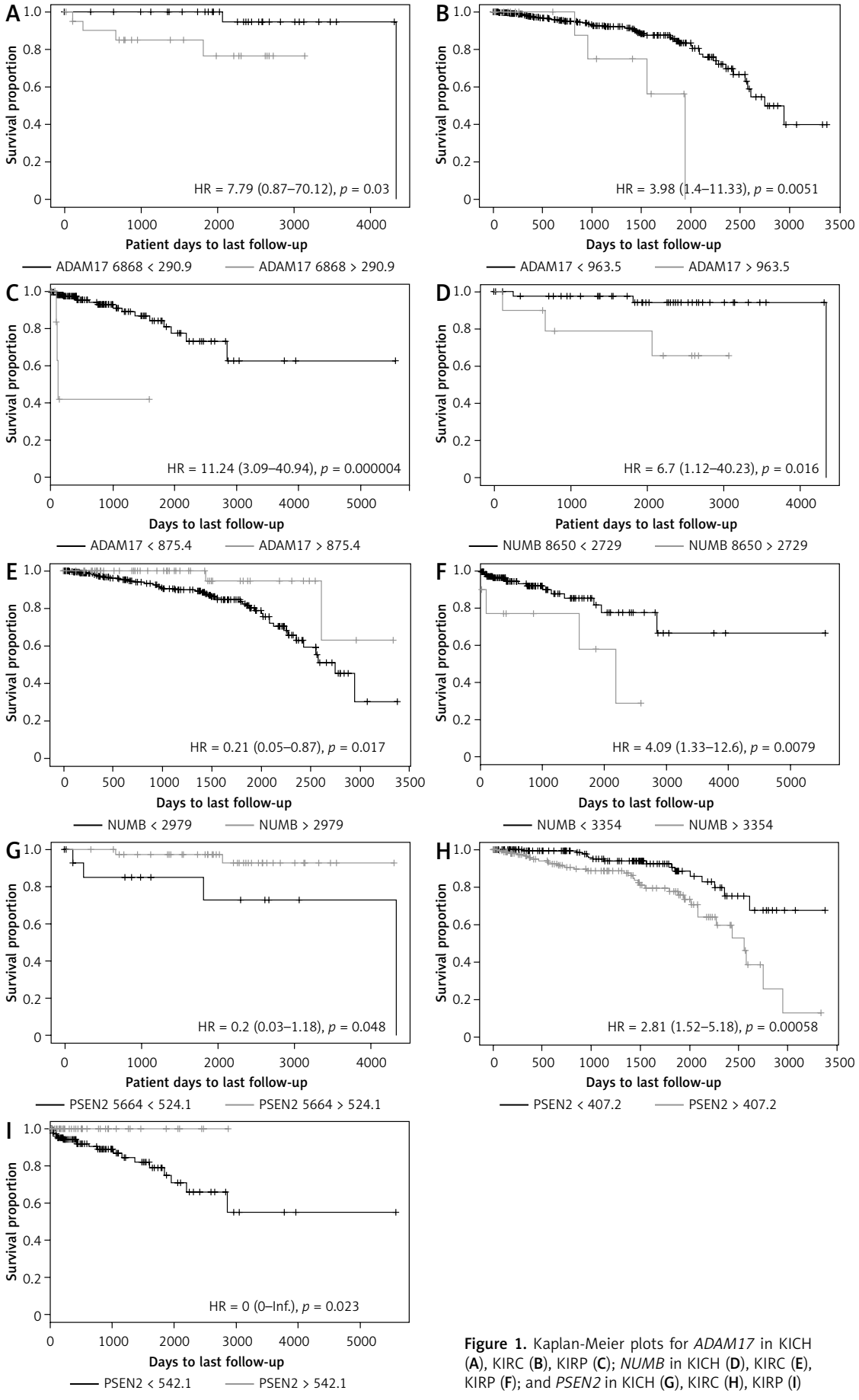

Figure 1. Kaplan-Meier plots for *ADAM17* in KICH (**A**), KIRC (**B**), KIRP (**C**); *NUMB* in KICH (**D**), KIRC (**E**), KIRP (**F**); and *PSEN2* in KICH (**G**), KIRC (**H**), KIRP (**I**)

Alzheimer's disease and many other dementia-associated disorders, but its function in renal cancer remains unclear [19]. Our findings show, for the first time, that elevated expression of *PSEN2* has a favourable effect in KICH and KIRP patients, while lowered *PSEN2* expression correlated with better prognosis in KIRC. These data suggest that conversely to *NUMB*, *PSEN2* may possibly function as a tumour suppressor in KICH and KIRP, and as an oncogene in KIRC. Differences in the favourable and unfavourable expression of *NUMB* and *PSEN2* in KIRC, KIRP and KIRP could serve as predictive factors distinguishing the KIRC subtype from two other types of renal cancer.

In addition to *ADAM17*, *NUMB* and *PSEN2*, which are significantly correlated with all types of renal cancer examined in our study, several members of the Notch pathway were associated with specific subtypes. Precisely, better prognosis in KIRC is characterized by low expression of *ADAM10* and *HES1* and high expression of *PSEN1*. Decreased expression of *HES5* and *JAG1* indicates better prognosis for KIRP patients and lowered expression of *NOTCH2* for KICH patients. The data would seem to suggest that some of the Notch pathway members are uniquely associated with particular subtypes of renal cancer.

In conclusion, our findings indicate that the expression profiles of Notch pathway members have a significant influence on DFS in renal carcinoma. As *NUMB* and *PSEN2* have contrasting effects on KIRC, KIRP and KICH, they could serve as prediction factors distinguishing these three subtypes. Moreover, the expression of particular genes may be used to predict the prognosis of relapse of the disease in patients with each subtype of renal cancer. Taken together, our results suggest that members of the Notch signalling pathway have great predictive value and they may serve as novel prognostic biomarkers in KIRC, KIRP and KICH; however, more studies are needed to confirm our results.

Acknowledgments

Dorota Jędroszka and Magdalena Orzechowska are co-first authors.

This work was funded by the Medical University of Lodz (grant no. 503/0-078-02/503-01-003).

Conflict of interest

The authors declare no conflict of interest.

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