Predictive values of Notch signalling in renal carcinoma

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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 $\gamma$-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 RBPJ 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 tumorigenesis. 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 mRNSeq 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 KIRC (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 KICH (70–75%), and the remainder comprising KIRC (about 10 % of cases) and KIRP (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-
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Though 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 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.
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 |
| Hess1 | 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.

Table: Statistics for DFS analysis. The table shows the gene names, cut-off values, number of patients in low and high expression groups, hazard ratios (HR), and P-values for DFS analysis. The cut-off values are determined for different genes in various tumor types, with corresponding numbers of patients in low and high expression groups and associated hazard ratios and P-values. The analysis suggests a relationship between gene expression and DFS prognosis, with notable findings for genes such as NUMB in KIRC and KIRP, indicating a potential dual role in tumorigenesis as a suppressor in KIRC and an oncogene in KICH and KIRP. Presenilin 2 (PSEN2) is also highlighted, being a member of the γ-secretase complex involved in NOTCH extracellular truncation (NEXT) [5].

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.

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Conflict of interest

The authors declare no conflict of interest.

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