Prognostic role of the beta-2 adrenergic receptor in clear cell renal cell carcinoma

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ABSTRACT

The beta-2 adrenergic receptor (ADRB2) regulates the proliferation, apoptosis, angiogenesis, migration, and metastasis of cancer cells. However, its function in the progression of clear cell renal cell carcinoma (ccRCC) is unknown. Here, we report that ADRB2 can be a novel prognostic factor for patients with ccRCC. The differential expression of ADRB2 in low-stage (stages I and II), high-stage (stages III and IV), low-grade (grades I and II), and high-grade (grades III and IV) ccRCC was identified in cohorts of patients from The Cancer Genome Atlas and the International Cancer Genome Consortium. We evaluated ADRB2 expression as a prognostic factor using the Kaplan-Meier survival curve, multivariate analysis, time-dependent area under the curve (AUC) of Uno’s C-index, and AUC of the receiver operating characteristics (ROC) at five years. Kaplan-Meier analysis revealed that reduced ADRB2 expression is associated with poor prognosis in ccRCC patients. Analysis of C-indices and AUC-ROC further confirmed this result. Moreover, multivariate analysis confirmed the prognostic significance of ADRB2 expression. Collectively, these findings suggest that ADRB2 is a potential prognostic factor for ccRCC.

Introduction

Clear cell renal cell carcinoma (ccRCC) is the most prevalent subtype of kidney cancer and approximately 30% of kidney cancer patients present with metastasis (Nickerson et al. 2008). In addition, approximately 30% of ccRCC patients have been diagnosed with advanced disease (Karakiewicz et al. 2007). Current therapeutic treatments against renal cancer are not sufficiently effective; therefore, novel biomarkers for ccRCC that could provide prognostic information for clinical use are required. Moreover, prognostic biomarkers for ccRCC have been investigated in cohorts of patients from The Cancer Genome Atlas (TCGA) (Cerami et al. 2012; Cancer Genome Atlas Research et al. 2013) and the International Cancer Genome Consortium (ICGC) (International Cancer Genome Consortium et al. 2010).

Beta-adrenergic receptors (βARs) are G protein-coupled receptors that regulate various cellular processes, including proliferation, invasion, and activation of the immune response (Barron et al. 2012). βARs are expressed on tumor cells and stromal cells in the tumor microenvironment (Sloan et al. 2010; Powe et al. 2011), and stress-induced βAR activation recruits immune cells to primary tumors (Sloan et al. 2010). Moreover, the activation of βARs can reduce tumor cell proliferation and primary tumor growth in vivo (Carie and Sebti 2007). The beta-2 adrenergic receptor (ADRB2) is the most abundant receptor for sympathetic signaling in prostate luminal cells (Braadland et al. 2014). ADRB2 expression was decreased during prostate cancer metastasis (Yu et al. 2007). However, the clinical and prognostic significance of ADRB2 in ccRCC remain unknown. In this study, we present the first data on ADRB2 expression in cohorts of patients with well-defined primary ccRCC from TGCA and ICGC and ADRB2 can be an important prognostic factor of ccRCC.

Materials and methods

Patient data acquisition and statistical analysis

The clinical and genomic data were acquired from TCGA and the ICGC data portal (dcc.icgc.org) on March 2018.
Samples with insufficient survival data were excluded, as previously described (Han et al. 2018; Ha et al. 2019). Overall survival (OS) prediction and associated statistical analyses were performed using R software version 3.5.0 (The R Foundation for Statistical Computing). The following statistical methods were used for analyses: (1) Uno’s C-index, (2) area under the curve (AUC) values at five years, and (3) p-value from log-rank test to evaluate the accuracy of the discrimination, as described previously using ‘survival’ and ‘survAUC’ R packages (Cho et al. 2018; Han et al. 2018). The C-index is a well-known parameter of the fit of a survival model, in continuous time, within a clinical study (Uno et al. 2011; Kim, Jeong, Pak, Goh, et al. 2017; Kim, Jeong, Pak, Han, et al. 2017). In the Kaplan-Meier analyses, we determined the optimal cut-off value (TCGA: 31.5365 and ICGC: 0.732) that had the maximal Uno’s C-index by five-fold cross-validation (Table 1) (Cho et al. 2018; Han et al. 2018; Ha et al. 2019). Univariate and multivariate Cox regression analysis was performed to assess the effect of ADRB2 expression as a categorical value on prognosis, along with other clinical variables (Table 2).

Table 1. C-index and area under the curve (AUC) values for ADRB2 in the specified categories in TCGA or ICGC cohorts.

| Category          | TCGA C-index | TCGA AUC value at 5 years | ICGC C-index | ICGC AUC value at 5 years |
|-------------------|-------------|--------------------------|-------------|--------------------------|
| All patients      | 0.605       | 0.677                    | 0.588       | 0.642                    |
| Stages I & II     | 0.543       | 0.442                    | 0.531       | 0.521                    |
| Stages III & IV   | 0.577       | 0.758                    | 0.572       | 0.777                    |
| Grades I & II     | 0.521       | –                        | 0.502       | –                        |
| Grades III & IV   | 0.600       | –                        | 0.602       | –                        |

TCGA: The Cancer Genome Atlas; ICGC: International Cancer Genome Consortium.

**Results**

**Downregulation of ADRB2 in high-stage and high-grade patients with ccRCC**

In total, 446 patients from TCGA and 91 from the ICGC were included in this study. Patient information is summarized in Table 3. ADRB2 expression was compared between low-stage (stages I and II) and high-stage (stages III and IV) cohorts of patients with ccRCC from TCGA and ICGC, and between low-grade (grades I and II) and high-grade (grades III and IV) cohorts of patients with ccRCC from TCGA, respectively. ADRB2 expression in the low-stage and low-grade ccRCC cohorts was considerably higher than that in the high-stage and high-grade cohorts (Figure 1).

![Figure 1](image_url)

**The prognostic value of ADRB2 expression in ccRCC patients**

To evaluate the prognostic value of ADRB2 in ccRCC, we analyzed Kaplan-Meier curves for ADRB2 gene expression and OS in TCGA (Figure 2) and ICGC (Figure 3) cohorts. Low expression of ADRB2 correlated with significantly shorter OS than did the high expression of ADRB2 in TCGA (Figure 2) and ICGC cohorts (Figure 3). The prognostic value was further confirmed using multivariate analysis (P < 0.001 and P = 0.003 for TCGA and ICGC, respectively, in Table 2).

To assess the validity of ADRB2 expression as a prognostic factor for ccRCC, we assessed Uno’s C-index from time-dependent AUC analysis and AUC at five years for receiver

**Table 2. Univariate and multivariate analysis of overall survival in each cohort (P < 0.05, ** P < 0.01, *** P < 0.001).**

| Parameters   | TCGA | ICGC | Univariate analysis | Multivariate analysis |
|--------------|------|------|---------------------|-----------------------|
|               | P    | HR   | 95 CI               |                       |
| TCGA          |      |      |                     |                       |
| ADRB2         | <0.001*** | 0.458 | 0.324 | 0.638               | <0.001*** | 0.532 | 0.375 | 0.755 |
| Age           | <0.001*** | 1.033 | 1.018 | 1.047               | <0.001*** | 1.030 | 1.015 | 1.046 |
| Stage (I, II vs. III, IV) | <0.001*** | 3.478 | 2.474 | 4.888               | <0.001*** | 2.730 | 1.903 | 3.917 |
| Gender (Female vs. Male) | 0.333 | 0.850 | 0.612 | 1.181               | 0.569 | 0.904 | 0.640 | 1.278 |
| Grade (I, II vs. III, IV) | <0.001*** | 2.247 | 1.572 | 3.212               | 0.040* | 1.486 | 1.019 | 2.168 |
| ICGC          |      |      |                     |                       |
| ADRB2         | <0.001*** | 0.299 | 0.146 | 0.614               | 0.003**  | 0.302 | 0.137 | 0.666 |
| Age           | 0.109 | 1.031 | 0.993 | 1.071               | 0.157 | 1.028 | 0.990 | 1.067 |
| Stage (I, II vs. III, IV) | <0.001*** | 4.796 | 2.264 | 10.16               | <0.001*** | 4.282 | 1.978 | 9.269 |
| Gender (Female vs. Male) | 0.863 | 1.066 | 0.517 | 2.194               | 0.758 | 1.130 | 0.518 | 2.466 |

TCGA: The Cancer Genome Atlas; ICGC: International Cancer Genome Consortium; ADRB2: Beta-2 adrenergic receptor.
operating characteristics (ROCs) in TCGA and ICGC cohorts (Figure 4). ADRB2 exhibited high C-index values in the two independent cohorts (TCGA: 0.605 and ICGC: 0.677; Figure 4A and Table 1). The five-year ROC graphs revealed high AUC values in TCGA and ICGC cohorts (TCGA: 0.588 and ICGC: 0.642; Figure 4B and Table 1).

**Discussion**

In this study, we identified ADRB2 expression as a prognostic factor for ccRCC, and demonstrated that reduced expression of ADRB2 is associated with poor patient prognosis. The current therapeutic treatment of ccRCC has a low rate of success (Subramanian and Haas 2018). Although there are many treatment options for ccRCC, surgical intervention is the most effective method to treat clinically localized ccRCC. Despite the availability of advanced surgical and medical techniques, ccRCC recurrence and metastasis rates remain high because of micro-environmental changes (Subramanian and Haas 2018; Wang et al. 2018). Transcriptome-based prognostic factors have been identified in many cancers, some of which have shown a sufficiently satisfactory outcome based on clinical guidelines.
Veer et al. 2002; Paik et al. 2004; Nault et al. 2013; Kim, Jeong, Pak, Goh, et al. 2017; Kim, Jeong, Pak, Han, et al. 2017). Therefore, novel molecular markers can be used in combination with current staging systems.

In summary, the main purpose of our study was to expand the foundation of precision medicine by analyzing big genome data. Our results showed that ADRB2 expression is inversely correlated with patient prognosis in both examined cohorts. Although there are limitations in transcriptome-based studies of ADRB2, we believe that there is sufficient evidence to suggest that ADRB2 can act as a prognostic biomarker in ccRCC.

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