Long intergenic non-coding RNA, regulator of reprogramming (LINC-ROR) over-expression predicts poor prognosis in renal cell carcinoma

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Abstract

Introduction: Long intergenic non-coding RNA, regulator of reprogramming (LINC-ROR) is a newly identified cytoplasmic long non-coding RNA (lncRNA) implicated in cell longevity and apoptosis. We aimed in the current work for the first time to investigate the association of the expression profiles of LINC-ROR and three stem-related transcriptional factors with clinicopathological data and their impact on renal cell carcinoma (RCC) progression in a sample of RCC patients.

Material and methods: Expression levels of LINC-ROR and stemness-related factors: SOX2, NANOG, and POU5F1 were detected in 60 formalin-fixed, paraffin-embedded tissues, and their paired adjacent non-cancer tissues (n = 60) by using real-time qRT-PCR analysis. Additionally, the expression profiles were compared with the available clinicopathological features.

Results: The genes studied were markedly up-regulated in RCC (medians and interquartile ranges were 30.3 (1.84–235.5), 10.2 (1.84–53.9), 5.39 (0.94–23.5), and 12.5 (1.61–43.2) for LINC-ROR, SOX2, NANOG, and POU5F1, respectively) relative to paired non-cancer tissue. High expression levels were associated with poor prognosis in terms of tumour undifferentiation (for LINC-ROR, SOX2, and NANOG), lymph node infiltration (for SOX2), post-operative recurrence (for LINC-ROR and SOX2), and shorter overall survival (OS) and progression-free survival (for all genes studied). The best curve for OS prediction was constructed with LINC-ROR data (area under the receiver operating characteristic curve (AUC) = 0.804 at a cut-off value of 72.7, sensitivity 78.9%, and specificity 80.5%).

Conclusions: Collectively, aberrant LINC-ROR and pluripotent gene expression may be recognised as prognostic markers for RCC. Future functional studies are highly recommended to validate the study findings.

Key words: renal cell carcinoma, long intergenic non-coding RNA, regulator of reprogramming, SOX2, NANOG, POU5F1.
Introduction

Renal cell carcinoma (RCC), which arises from renal tubular epithelial cells, accounts for 2–3% of all cancers worldwide [1]. There has been a global rise in RCC incidence in past decades, and up to 35% of RCC patients present with metastasis [2]. Therefore, discovering biomarkers for early detection and better prognostic stratification of RCC has emerged as a noteworthy target for future investigations.

According to the World Health Organisation (WHO), there are three major histopathological RCC types: clear cell RCC (ccRCC), papillary cell RCC (pcRCC), and chromophobe RCC (chRCC) [3]. Each RCC subtype is known to display unique pathological characteristics, distinct genetic alterations, and different disease outcomes [1]. The interplay of the genetic mechanisms that affect RCC pathogenesis is still poorly understood. The use of integrated molecular analyses enables a deeper understanding of the RCC molecular landscape, resulting in the development of more effective targeted cancer therapies as an extension of existing modalities [4, 5].

Cancer has been found to histologically mimic embryonic tissue [6]. Aggressive cancer cells exhibit phenotypic traits remarkably similar to those of pluripotent stem cells; both have high proliferation rates and a substantial ability to self-renew by bypassing senescence [7]. Accumulating evidence has demonstrated the convergence of tumorigenic signalling and developmental pathways [8]. Since the introduction of induced pluripotent stem cell (iPSC) generation mediated by transcription factor (TF) reactivation, studies have been directed towards identifying the differential expression profiles altered during pathological nuclear reprogramming in cancer [7]. SOX2, NANOG, and POU5F1 (also known as OCT3/4) are three pluripotency-associated TFs that are well known to be up-regulated in various cancer types through modulation of apoptotic signalling pathways [9]. Notably, modifying the expression of pluripotency-associated genes in animal models has generated epithelial-derived tumours in multiple tissues with altered spatial and temporal gene expression [10].

Recent evidence has suggested complex networking between TFs, chromatin organisation, and non-coding RNAs that regulate cell fate and differentiation. Several long intergenic non-coding RNAs (lincRNAs) have also been shown to be associated with pluripotency [11]. LincRNA expression has been shown to be highly correlated with the expression of stem cell-related TFs [12]. Binding sites for pluripotency-related genes have been identified within the promoters of these long non-coding RNAs (lincRNAs) [13]. In vitro silencing of lincRNAs has been shown to induce cells to exit the pluripotent state and altered gene expression patterns [14]. In 2010, Loewer et al. [11] identified lincRNA, regulator of reprogramming (LINC-ROR or ROR) in human fibroblasts and CD34-positive blood cells during iPSC generation. Furthermore, in vitro and in vivo studies have determined the critical role of ROR in modulating cell proliferation, apoptosis, and chemosensitivity in cancer [15–17].

To the best of our knowledge, no clinical studies have been conducted exploring the role of ROR in RCC patients to validate the aforementioned in vitro and in vivo results. In the current study, we aimed to analyse the transcriptomic signature profiling of LINC-ROR and three of the main pluripotency-related genes (SOX2, NANOG, and POU5F1) in RCC patients to assess their clinical utility as prognostic biomarkers.

Material and methods

Case selection and pathological assessment

In total, 120 formalin-fixed, paraffin-embedded (FFPE) specimens were analysed, including 60 RCC
samples (30 ccRCC, 15 pcRCC, and 15 chRCC) and their paired adjacent non-cancer tissues. Figure 1 shows the flow chart of the current study case selection. All specimens were obtained after radical or partial nephrectomy, and cases with needle biopsies were excluded. All retrieved cases were archived in the Department of Pathology, Suez Canal University between 2011 and 2016. Patient data were obtained from medical records. Missing data were directly retrieved from the patients. Patient follow-ups were performed until December 2016 and ranged from seven to 32 months. Postoperative recurrence was calculated, and survival times were estimated from the date of nephrectomy until patient death or the endpoint of follow-up. The study was conducted following the ethical and legal standards adopted by the Declaration of Helsinki. Approval was obtained from the Medical Research Ethics Committee of the Faculty of Medicine, Suez Canal University. Consent was directly obtained from patients as a routine measure for sample archiving.

**Histopathological examination**

Histological examination, nuclear grading, and tumour staging of RCC samples were based on haematoxylin and eosiin staining. Blind assessments were made without prior knowledge, according to the internationally standardised protocols we mentioned in our previous work [18]. Most of the RCC cases were the clear cell type and contained very little delicate vascular stroma. The papillary and chromophobe types also had only a little amount of stroma, and thus it was unlikely that it affected accurate determination of the expression of the genes assessed in this study.

**Gene expression profiling**

Total RNA was purified from the FFPE sections using a Qiagen RNeasy FFPE Kit (Cat # 74404, Qiagen, Hilden, Germany) following the manufacturer's protocol. A short incubation of each sample with proteinase K at a higher temperature, as recommended, partially reverses formalin crosslinking of the released nucleic acids, improving RNA yield and quality as well as RNA performance in downstream assays. All samples were subjected to treatment with RNase-free DNase I for 2 h at 37°C to eliminate all genomic DNA, including very small DNA fragments that are often present in FFPE samples after prolonged formalin fixation and/or long storage times. At an absorbance ratio of 260/280 nm, RNA concentration and purity were assessed by a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE), followed by agarose gel electrophoresis check for RNA integrity. The range of the extracted RNA was (20–65 ng/μl). Reverse transcription was performed with a high-capacity cDNA reverse transcription kit (Part No. 4374966, Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) using a Mastercycler Gradient Thermocycler (Eppendorf, Hamburg, Germany). Detection of LINC-ROR, SOX2, NANOG, and POU5F1 gene expression and that of the endogenous control GAPDH was performed using real-time quantitative reverse transcriptase polymerase chain reaction (qRT-PCR). TaqMan assays were used for the pluripotent genes (Applied Biosystems, Thermo Fisher Scientific Inc., assay ID Hs01053049_s1 for SOX2, Hs02387400_g1 for NANOG, Hs03005111_g for POU5F1, and Hs02786624_g1 for GAPDH) as previously described in detail [19]. Quantification of LINC-ROR was achieved using SYBR Green with primers for exon 4 (F: GCCTGAGAGTTGGCATGAAT and R: AAAACCTCACCCTCCATGCG) [20]. The Minimum Information for Publication of Quantitative Real-Time PCR Experiments (MIQE) guidelines were followed for all PCR reactions. Gene expression in randomly selected study samples (10%) was re-evaluated in separate runs to test the reproducibility of the qPCR analysis, and the results showed very close cycle of quantification (Cq) values with low standard deviations.

**Statistical analysis**

Data analyses were conducted using the R packages SPSS version 22 and GraphPad Prism version 7. Multivariate analysis was conducted using PCORD version 5.0. The relative expression levels were calculated using the delta-delta threshold d cycle method. The Wilcoxon matched-pair signed-rank test was carried out to compare the expression level between cancer samples and their corresponding adjacent non-cancer tissues. Receiver operating characteristic (ROC) curves and the area under the ROC curves (AUCs) were determined to identify the clinical utility of the expressed genes. Ordination analysis and two-way agglomerative hierarchical clustering were performed for data exploration. Spearman’s correlation analysis, chi-square (χ²), Fisher’s exact, Mann-Whitney U (MW), and Kruskal-Wallis (KW) tests were applied when appropriate. A two-tailed p-value was considered significant at values < 0.05. Binary logistic and linear regression methods (Enter and Stepwise models) were applied to determine predictors for recurrence and overall survival (OS). The influence of covariates, such as age, gender, grade, tumour type, size, and gene profile, on OS was analysed. Kaplan-Meier analysis and the Cox proportional hazard model were performed to assess survival rates.

**Results**

**Characteristics of the study patients**

The baseline features of RCC patients are shown in Table I. Overall, 21 female and 39 male
RCC patients were included in the study. Their ages ranged from 48 to 79 years (mean ± standard deviation of 58.6 ±6.31). Notably, in patients with solitary tumours (76.7%), the left kidney was more frequently affected (63.3%). Despite the lack of lymph node metastases in nearly two-thirds of the specimens (65%), most samples showed moderate/poor differentiation (85%). Recurrence occurred in approximately one-fourth of patients (26.7%) after a mean duration of 4 ±1.7 months. Postoperative disease-free survival ranged from 2 to 32 months. The OS rate...
Gene expression profiling demonstrated over-expression of the LINC-ROR, SOX2, NANOG, and POU5F1 genes in tumour specimens compared with those in their paired non-cancer tissues (all \( p < 0.001 \)). Medians and interquartile ranges of the relative expression levels in RCC were 30.3 (1.84–235.5) for LINC-ROR, 10.2 (1.84–53.9) for SOX2, 5.39 (0.94–23.5) for NANOG, and 12.5 (1.61–43.2) for POU5F1 (Figure 2). Stratified analyses by age and gender showed similar up-regulation (all \( p \)-values < 0.001), with marked over-expression of LINC-ROR in female patients less than 60 years of age at disease onset. The correlation matrix showed a significant positive relationship between LINC-ROR and both SOX2 (\( r = 0.280, p = 0.045 \)) and NANOG (\( r = 0.312, p = 0.015 \)).

**Prognostic value of gene expression analyses**

Univariate analysis revealed marked associations between LINC-ROR, SOX2, and NANOG expression levels and undifferentiated tumours (\( p = 0.010, 0.046, \) and 0.042, respectively). SOX2 up-regulation was linked to lymph node infiltration (\( p = 0.047 \)). Moreover, patients with higher LINC-ROR and SOX2 levels experienced post-operative recurrence (\( p = 0.003 \) and 0.035). The expression of all genes presented significant associations with shorter PFS and low OS (all \( p < 0.05 \)) (Table VI). Similar results were found in correlation analyses between gene expression levels and the clinicopathological findings (Table IV).

The prognostic performance of the four genes in predicting OS in RCC patients was assessed using ROC analyses (Figure 3). In single-gene analyses, LINC-ROR data elicited the best curve (AUC = 0.804 at a cut-off value of 72.7; sensitivity 78.9%, specificity 80.5%) followed by POU5F1 (AUC = 0.748 at a cut-off value of 72.7; sensitivity 73.3%, specificity 75.6%) and SOX2 (AUC = 0.714 at a cut-off value of 75.5; sensitivity 73.3%, specificity 75.6%) followed by NANOG (AUC = 0.748 at a cut-off value of 72.7; sensitivity 78.9%, specificity 80.5%). Despite the good performance of the combined gene analyses, with AUC values ranging from 0.675 to 0.755, the AUC of LINC-ROR remained the best (Table V).

Logistic regression analyses revealed that both lymph node infiltration and LINC-ROR expression acted as recurrence predictors in RCC patients (\( p = 0.007 \) and 0.047, respectively; Table VI). Furthermore, linear regression analyses demonstrated that LINC-ROR was an independent predictor for OS (R = 0.443, \( R^2 = 0.192, p < 0.001 \)).

**Survival analysis**

Analyses of different influencing factors on the survival time of RCC patients are demonstrated in Table VII. Cox regression showed that LINC-ROR, POU5F1, and recurrence were risk factors that predicted OS (hazard ratio (HR) = 4.21, 95% confi-
Table III. Univariate analysis for association between gene profile and the clinicopathological features in RCC patients

| Parameter      | LINC-ROR | SOX2   | NANOG  | POU5F1  |
|----------------|----------|--------|--------|---------|
|                | Expression | P-value | Expression | P-value | Expression | P-value | P-value |
| Age: ≤ 60 years| 28.6 (1.8–65) | 0.178   | 10 (0.9–23.5) | 0.563     | 7.9 (1.9–44.1) | 0.947    |
|                | 7.19 (1.3–34) | 3.9 (0.7–30.5) | 14 (1.4–41.3) |         |           |         |
| Age: ≥ 60 years| 32 (4.8–214)  | 0.62    | 5.6 (0.5–23.1) | 0.871     | 12.3 (1.5–42) | 0.625    |
|                | 12 (1.7–56.8) | 2 (0.2–14) | 10 (1.7–43) |         |           |         |
| Gender: Female | 39 (11.2–575) | 4.7 (1.3–38) | 14.3 (2.8–46) |         |           |         |
|                | 8.6 (1.7–40.9) | 12 (1.7–61) | 14 (1.5–45) |         |           |         |
| Gender: Male   | 13 (1.3–152)  | 16 (1.6–61) | 12 (1.7–61) | 3 (1.2–34) |         |         |
| HPD: ccRCC     | 8 (1.9–45)    | 4.8 (0.2–15) | 14 (1.5–45) |         |           |         |
|                | 25 (1.0–65)   | 15 (1.6–61) | 12 (0.7–42) |         |           |         |
| HPD: chRCC     | 12 (1.7–61)   | 4.7 (0.2–14) | 10 (1.7–43) |         |           |         |
| Side: Right    | 22 (2.2–169)  | 14 (3.1–56) | 4.6 (0.4–50) | 3 (1.2–34) |         |         |
|                | 36 (1.6–239)  | 10 (1.6–16) | 5.9 (1.3–16) | 16 (2.3–44) |         |         |
| Side: Left     | 21 (2.2–169)  | 14 (3.1–56) | 4.6 (0.4–50) | 3 (1.2–34) |         |         |
| Number: Unifocal | 46 (2.2–165)  | 9.4 (0.4–45) | 4.9 (0.4–20.9) | 13 (1.5–44) |         |         |
|                | 17 (3.9–85)   | 13 (1.8–42.6) | 73 (1.6–23) |         |           |         |
| Number: Multifocal | 14 (1.4–985) | 25 (1.0–65) | 15 (1.6–61) | 12 (0.7–42) |         |         |
| Grade: Grade 1 | 3.3 (0.6–105) | 6.5 (0.004–21) | 12.3 (1.7–73) |         |           |         |
|                | Grade 2      | 7.1 (0.63–30) | 2.8 (0.21–9.3) | 9.9 (1.4–32.2) |         |           |         |
|                | Grade 3      | 35.7 (3.9–65) | 12.3 (2.0–61) | 15 (2.5–44.3) |         |           |         |
| T: T1          | 2.1 (0.19–27) | 6.5 (0.23–33.6) | 12.6 (4.6–45.2) |         |           |         |
|                | T2           | 12 (4.7–45.3) | 4.6 (1.1–11.3) | 12.3 (1.4–44.2) |         |           |         |
|                | T3           | 49 (3.7–86.4) | 8.3 (1.1–61.8) | 10.5 (1.6–17.9) |         |           |         |
| LN: Negative   | 7.5 (0.2–36.6) | 5.1 (0.51–23) | 12.6 (1.5–44.3) |         |           |         |
|                | Positive     | 29.2 (3.8–64) | 6.2 (1.0–30.1) | 7.9 (1.5–27.7) |         |           |         |
| Recurrence:    | 6.1 (0.6–45.7) | 4.6 (0.3–19) | 11.4 (1.4–35) |         |           |         |
|                | Positive     | 36.2 (17.60.4) | 12.4 (1.3–38) | 23.7 (4–61) |         |           |         |
| PFS: ≤ 12 m    | 37.6 (13.9–64) | 14 (3.7–61.8) | 17.3 (3.9–50.5) |         |           |         |
|                | ≤ 24 m       | 6.8 (0.2–446) | 4.6 (0.19–12) | 15.2 (1.3–39) |         |           |         |
|                | ≤ 36 m       | 3.5 (1.05–4.1) | 2.7 (1.5–10.2) | 1.4 (0.3–1.5) |         |           |         |
| OS: ≤ 12 m     | 36.6 (12–65)  | 106 (4.6–61) | 15.2 (4.1–49) |         |           |         |
|                | ≤ 24 m       | 7.5 (0.2–48) | 3.9 (0.1–12) | 16.3 (1.2–40) |         |           |         |
|                | ≤ 36 m       | 3.5 (1.1–4.1) | 2.7 (1.5–10) | 1.4 (0.3–1.5) |         |           |         |

Data are presented as median (quartiles). HPD – histopathological diagnosis, ccRCC – clear cell renal cell carcinoma, pRCC – papillary cell RCC, chRCC – chromophobe RCC, T – tumour size, LN – lymph node, PFS – progressive-free survival, OS – overall survival. Mann-Whitney U and Kruskal-Wallis tests were used. *Values are statistically significant at p < 0.05.

Confidence interval (CI): 1.13–15.6, p = 0.031; HR = 3.80, 95% CI: 1.55–9.30, p = 0.003; and HR = 4.94, 95% CI: 1.98–12.3, p = 0.001, respectively) in RCC patients. Using Kaplan-Meier analysis, a comparison of survival curves of patients with low versus high LINC-ROR, SOX2, NANOG, and POU5F1 gene expression revealed significant differences in OS (p = 0.018, 0.008, 0.028, and 0.001, respectively), which suggested that high expression was associated with poor survival (Figure 4). Similar find-
Table IV. Correlation analysis between gene expression and the clinicopathological characteristics in RCC patients

| Parameter | LINC-ROR | SOX2 | NANOG | POU5F1 |
|-----------|----------|------|-------|--------|
| Age       | r        | P-value | r    | P-value | r    | P-value | r    | P-value |
| Gender    | -0.002   | 0.987 | -0.189 | 0.148 | 0.009 | 0.949 | -0.031 | 0.812 |
| HPD       | -0.245   | 0.059 | 0.065 | 0.624 | -0.021 | 0.872 | -0.064 | 0.629 |
| Side      | 0.014    | 0.916 | -0.043 | 0.745 | -0.045 | 0.733 | 0.194 | 0.138 |
| Number    | 0.100    | 0.447 | 0.190 | 0.146 | 0.134 | 0.306 | -0.092 | 0.484 |
| Grade     | 0.387    | 0.002 | 0.314 | 0.014 | 0.265 | 0.041 | 0.077 | 0.558 |
| Tumour size | 0.185  | 0.158 | 0.304 | 0.018 | -0.009 | 0.946 | -0.141 | 0.282 |
| LN        | 0.152    | 0.245 | 0.258 | 0.046 | 0.043 | 0.742 | -0.100 | 0.448 |
| Recurrence | 0.383  | 0.003 | 0.274 | 0.034 | 0.139 | 0.289 | 0.190 | 0.145 |
| PFS       | -0.577   | < 0.001 | -0.511 | < 0.001 | -0.339 | 0.008 | -0.419 | 0.001 |
| OS        | -0.576   | < 0.001 | -0.488 | < 0.001 | -0.403 | 0.001 | -0.464 | < 0.001 |

Data are shown as a correlation coefficient (r) and its p value. HPD – histopathological diagnosis, LN – lymph node, PFS – progressive-free survival, OS – overall survival. Spearman's rank correlation test was used. Bold values are statistically significant at p < 0.05.

**Discussion**

A complex network of TFs, non-coding RNAs, and signalling transducers drives cancer. Unravelling the interactions between these molecular cancer players would pave the road towards a better understanding of cancer development and progression.

Currently, marked up-regulation of LINC-ROR, SOX2, NANOG, and POU5F1 genes was found in cancer samples compared with the adjacent non-cancer tissues. Additionally, LINC-ROR expression levels were positively correlated with SOX2 and NANOG levels. These results were consistent with previous studies [14, 15]. Pluripotent TFs, which are often epigenetically maintained in a silent state, have been shown to be reactivated in several cancers, and their knockdown or suppression with microRNA or small interfering RNA transfection has been shown to hinder tumour progression, promote apoptosis, and improve chemosensitivity [21]. Interestingly, these TFs are now well known to operate in conjunction with non-coding RNAs. A few human IncRNAs may also be under the direct control of the core pluripotency TFs [22]. A recently identified cytoplasmic IncRNA, LINC-ROR, was reported to play a crucial role in the pluripotency maintenance [23]. The promoter of the LINC-ROR gene was found to contain binding sites for SOX2, NANOG, and POU5F1 [24]. Upon binding of TFs, LINC-ROR transcription is activated, whereas silencing of these proteins suppressed LINC-ROR expression through a regulatory feedback loop [25]. Similarly to our findings, LINC-ROR over-expression has also been observed in several cancers, including colorectal carcinoma [15] and breast cancer [25].

Another main finding of our study was the association of the four genes with poor prognosis. The
Kaplan-Meier curves and ROC analyses showed that the LINC-ROR, SOX2, NANOG, and POU5F1 expression profiles were associated with shorter survival times. RCC patients with high LINC-ROR and SOX2 levels had a higher recurrence rate after an average of 4 months post-nephrectomy. Notably, regression analyses confirmed that LINC-ROR was an independent predictor for recurrence and poor OS. In addition, LINC-ROR, SOX2, and NANOG expression showed a significant association with poor differentiation, whereas SOX2 was a poor marker for LN metastasis. In accordance with previous studies, melanoma and HCC cells expressing high POU5F1 and NANOG levels exhibited a more aggressive malignant phenotype [26]. NANOG expression has also been considered a prognostic biomarker for triple-negative breast cancer (TNBC) [27].

Recently, increasing attention has been focused on the contribution of LINC-ROR to cancer progression. LINC-ROR has been suggested to be a driving factor in tumorigenesis [24], as depicted in Figure 6. Increased LINC-ROR expression levels have been associated with differentiation, apoptosis, invasion, EMT, and metastasis in cancer [24, 28]. Up-regulation was recognised as a poor prognostic factor in colon cancer [29]. However, LINC-ROR silencing reduced the malig-
Table VII. Survival analysis of renal cell carcinoma patients

| Parameter       | Survival time, median (95% CI) [months] | Overall comparisons | Cox regression |
|-----------------|-----------------------------------------|--------------------|----------------|
|                 | Log rank | Breslow | Tarone-Ware | HR (95% CI) | Wald | P-value |
| Age:            |          |         |            |            |      |         |
| < 60 years      | 13 (9.8–6.1) | 0.088  | 0.054      | 0.061       | Ref. |         |
| ≥ 60 years      | 17 (14.9–19.0) | 0.98 (0.94–1.03) | 0.317 | 0.573 |      |         |
| Gender:         |          |         |            |            |      |         |
| Female          | 13 (8.51–17.4) | 0.127  | 0.077  | 0.095      | Ref. |         |
| Male            | 16 (13.3–18.6) | 0.59 (0.26–1.33) | 1.608 | 0.205 |      |         |
| HPD:            |          |         |            |            |      |         |
| ccRCC           | 15 (12.3–17.6) | 0.103  | 0.198      | 0.159      | Ref. |         |
| pRCC            | 17 (13.2–20.7) | 0.60 (0.25–1.44) | 1.307 | 0.253 |      |         |
| chRCC           | 15 (7.42–22.5) | 1.41 (0.62–3.11) | 0.740 | 0.390 |      |         |
| Side:           |          |         |            |            |      |         |
| Right           | 16 (12.3–19.6) | 0.703  | 0.489      | 0.539      | Ref. |         |
| Left            | 15 (12.9–17.0) | 1.06 (0.54–208) | 0.034 | 0.854 |      |         |
| Number:         |          |         |            |            |      |         |
| Unifocal        | 16 (14.3–17.6) | 0.461  | 0.291  | 0.350      | Ref. |         |
| Multifocal      | 13 (11.1–14.8) | 0.89 (0.38–2.10) | 0.063 | 0.802 |      |         |
| Grade:          |          |         |            |            |      |         |
| 1               | 16 (13.1–189) | 0.005* | 0.003*  | 0.003*     | Ref. |         |
| 2               | 18 (15.4–20.5) | 0.47 (0.17–1.30) | 2.101 | 0.147 |      |         |
| 3               | 12 (8.4–155) | 0.82 (0.22–2.98) | 0.089 | 0.765 |      |         |
| T:              |          |         |            |            |      |         |
| T1              | 16 (11.5–20.4) | 0.709  | 0.577  | 0.635      | Ref. |         |
| T2              | 16 (12.7–19.2) | 0.84 (0.40–1.77) | 0.195 | 0.659 |      |         |
| T3              | 13 (9.3–16.6) | 0.93 (0.26–3.34) | 0.011 | 0.917 |      |         |
| LN:             |          |         |            |            |      |         |
| Negative        | 16 (13.5–18.4) | 0.306  | 0.235  | 0.255      | Ref. |         |
| Positive        | 14 (11.3–16.6) | 0.83 (0.23–2.89) | 0.084 | 0.772 |      |         |
| Recurrence:     |          |         |            |            |      |         |
| Negative        | 17 (15.2–18.7) | < 0.001* | < 0.001* | < 0.001* | Ref. |         |
| Positive        | 10 (9.1–10.9) | 4.94 (1.98–12.3) | 11.782 | 0.001* |      |         |
| LINC-ROR:       |          |         |            |            |      |         |
| Low             | 22 (13.5–30.4) | 0.007* | 0.018*  | 0.012*     | Ref. |         |
| High            | 15 (12.9–17.0) | 4.21 (1.13–15.6) | 4.641 | 0.031* |      |         |
| SOX2:           |          |         |            |            |      |         |
| Low             | 20 (16.6–23.3) | 0.089* | 0.008*  | 0.018*     | Ref. |         |
| High            | 14 (12.1–15.8) | 2.57 (0.99–6.6) | 3.814 | 0.051 |      |         |
| NANOG:          |          |         |            |            |      |         |
| Low             | 18 (16.7–19.2) | 0.295  | 0.028*  | 0.081      | Ref. |         |
| High            | 14 (11.8–16.1) | 1.10 (0.50–2.41) | 0.065 | 0.799 |      |         |
| POUSF1:         |          |         |            |            |      |         |
| Low             | 22 (19.1–24.8) | 0.003* | 0.001*  | 0.001*     | Ref. |         |
| High            | 14 (12.1–15.8) | 3.80 (1.55–9.30) | 8.544 | 0.003* |      |         |

CI – confidence interval, HR – hazard ratio, Ref. – reference, HPD – histopathological diagnosis, ccRCC – clear cell renal cell carcinoma, pRCC – papillary cell RCC, chRCC – chromophobe RCC, T – tumour size, LN – lymph node, PFS – progression-free survival, OS – overall survival.

*Values are statistically significant at p < 0.05.
Long intergenic non-coding RNA, regulator of reprogramming (LINC-ROR) over-expression predicts poor prognosis in renal cell carcinoma

Figure 4. Kaplan-Meier curves comparing patient survival with different clinical variables (A – tumor grade, B – tumor size, C – LN infiltration, D – recurrence, E – LINC-ROR, F – SOX2, G – NANOG, H – POU5F1). Log-rank (Mantel-Cox) test was used for comparisons. The significance level was set at p < 0.05
Figure 5. Multivariate analysis cluster of RCC patients according to the combined transcriptomic gene signature and clinicopathological features. PC-ORD v5.0 was used for data exploration. Ordination graph constructed via Bray-Curtis analysis. The following setup parameters were adjusted. Distance method: relative Euclidean method; endpoint selection method: variance-regression; axis projection geometry: Euclidean; residual distances: Euclidean; score calculation by weighted averaging. Samples were scattered along multiple axes; axis 1 represents 30.1% of variance, whereas axes 2 and 3 account for 14.6% and 11% of clustering, respectively. The study population was clustered into three distinct groups according to survival time. Notably, most of the patients with poor survival (less than 1 year) manifested high levels of LINCROR. The same category of patients was associated with poor differentiation and recurrence.

Figure 6. The role of LINCROR in tumourigenesis and cancer progression.
Long intergenic non-coding RNA, regulator of reprogramming (LINC-ROR) over-expression predicts poor prognosis in renal cell carcinoma

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