Association of lncRNA and transcriptome intersections with response to targeted therapy in metastatic renal cell carcinoma

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Abstract. Long non-coding RNAs (lncRNAs) serve an important role in cancer progression and may be used as efficient molecular biomarkers. The present study aimed to identify lncRNAs associated with the response to the receptor tyrosine kinase inhibitor sunitinib and transcriptome profile and clinical features of metastatic renal cell carcinoma (mRCC). The gene expression of 84 cancer-associated lncRNAs in tumor and non-malignant tissue samples of 38 patients with mRCC was evaluated using quantitative PCR. In addition, the coding transcriptome was estimated using RNA sequencing in a subgroup of 20 patients and mRNA-lncRNA intersections were identified. In total, 37 and 13 lncRNAs were down- and upregulated, respectively, in tumor compared with non-malignant adjacent tissue samples. A total of 10 and 4 lncRNAs were up- and downregulated, respectively, in good responders to sunitinib compared with poor responders. High expression of HNF1A-AS1 and IPW lncRNAs was associated with prolonged progression-free survival of patients and a high expression of the TUSC7 lncRNA was associated with poor response and worse survival. Significant associations of dysregulated MEG3 and SNHG16 lncRNAs with expression of protein-coding genes representing various pathways, were identified. Furthermore, a significantly higher expression of *CLIP4* gene was observed in good responders. The present study revealed promising candidates for predictive and prognostic biomarkers with further therapeutic potential.

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Introduction

Renal cell carcinoma (RCC) is a urological malignancy with increasing incidence in countries with a high Human Development Index (HDI) (i.e. with a HDI of >0.8) (1,2). Clear cell RCC (ccRCC) is the predominant histological type, representing 75-80% of all cases (3). A total of ~20% of patients are initially diagnosed with metastatic ccRCC (mRCC) and ~20% of primary localized cases become metastatic during follow-up (4,5). The management of mRCC has markedly changed in recent years with the introduction of novel therapies leading to substantial improvements in the survival and quality of life of patients (6). Antiangiogenic tyrosine kinase inhibitors (TKIs), immune checkpoint inhibitors (ICIs) and their combination represent novel systemic therapies in mRCC (7,8). The expanding horizons of systemic therapies suitable for mRCC require predictive and prognostic molecular biomarkers for the selection of optimal therapy approaches for patients in personalized medicine.

Long non-coding RNAs (lncRNAs) have emerged as one of the key regulators of gene expression in cancer, and they may exhibit tumor-suppressive or oncogenic functions based on their interacting partners. Their expression is highly tissue- and condition-specific, and they have been found to be involved in various cancer-associated processes, including tumorigenesis, progression and metastatic spread (9). Thus, lncRNAs hold promise as novel biomarkers and therapeutic targets for cancer.

Previous studies have shown several differentially expressed lncRNAs in ccRCC tissue, as well as in ccRCC cell lines, including CRNDE (10), H19 (11), HOTAIR (12), TUG1 (13), MEG3 (14) and GAS5 (15) as summarized by Li *et al* (16). Moreover, MALAT1 (17), PVT1 (18), LINC00152 (19) and LUCAT1 (20) have been suggested as lncRNAs associated with poor prognosis and decreased overall survival (OS) in patients with ccRCC. A potential predictive role of several lncRNAs has previously been suggested in experimental studies (21-23). The lncRNA ARSR promotes resistance to sunitinib by serving as competing endogenous RNA (22), whereas high expression of lncRNA

SARCC increases its efficacy (23). In mRCC specifically, mostly protein-coding genes have been studied (24,25). Genes for transmembrane protein programmed death-ligand 1 and serine/threonine-protein kinase p21-activating kinase 1 serve a prognostic role in mRCC (26,27). Nevertheless, the association between lncRNA profiles and outcomes of patients with mRCC treated with a specific type of systemic therapy remains unclear.

The present study evaluated the expression profile of 84 cancer-associated lncRNAs in patients with mRCC treated with sunitinib as the first-line treatment. Differences in lncRNA expression levels between primary tumors and adjacent non-malignant tissue were analyzed. The present study also evaluated associations of lncRNA expression profile with clinical data, including objective response and patient survival. The role of clinically relevant lncRNAs in the context of mRNA transcriptome profile was investigated to reveal their mutual interactions and biological importance.

Materials and methods

Patients and tissue samples. The present study included 38 patients with mRCC treated with sunitinib (SUTENT®; Pfizer, Inc.) as first-line therapy at the Department of Oncology and Radiotherapeutics, University Hospital Pilsen (Pilsen, Czech Republic). Only patients with ccRCC histology and those with favorable or intermediate risk according to the Memorial Sloan-Kettering Cancer Center (MSKCC) prognostic model were included (28,29). All patients had distant metastases at the start of sunitinib therapy. Sunitinib was administered orally at the standard approved dosing (30) until disease progression, unacceptable toxicity or patient refusal. The clinical data, including baseline clinical characteristics, treatment course and outcomes, were obtained from medical records.

Physical examination and routine laboratory tests were performed every <6 weeks and CT was performed every 3-4 months during treatment with sunitinib. The objective response was assessed by an independent experienced radiologist using Response Evaluation Criteria in Solid Tumors version 1.1 (31). The objective response was classified in terms of complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD) (2).

Fresh-frozen tissue samples, including primary ccRCC tumor and adjacent non-neoplastic kidney tissue, were obtained during radical or cytoreductive nephrectomy surgery and were stored in RNAlater (cat. no. AM7020; Thermo Fisher Scientific, Inc.) at -80˚C until processing.

All patients signed an informed consent for participation. The Ethics Committee of the Faculty of Medicine and University Hospital Pilsen, Charles University (approval no. 302/2020) approved the informed consent form and study protocol for samples collected during the project. The present study was performed in accordance with the Declaration of Helsinki.

Baseline clinical data for all patients with mRCC included in the present study (n=38) and the subgroup (n=20) profiled by RNA sequencing (RNASeq) are summarized in Table I. The median age of patients at the time of sunitinib initiation was 64.5±8.8 years. The study included 26 male and 12 female patients. The majority of patients (>80%) belonged to the intermediate risk group according to the MSKCC criteria, and 50% of patients were evaluated as good responders to sunitinib as first-line systemic therapy. The median progression-free survival (PFS) and OS for the entire cohort were 7 and 36 months, respectively.

Isolation of total RNA and preparation of cDNA. Tissue samples were removed from RNAlater and ground to powder by mortar and pestle under liquid nitrogen. RNA was isolated using AllPrep DNA/RNA/Protein Mini kit (cat. no. 80004; Qiagen GmbH) according to the manufacturer's protocol. Total RNA was quantified with Quant-it™ RiboGreen RNA Assay kit (cat. no. R11490; Thermo Fisher Scientific, Inc.) on Microplate Reader-Infinite M200 (Tecan Group, Ltd.). RNA quality was determined by estimation of the RNA Integrity Number (32) with Agilent RNA 6000 Nano kit (cat. no. 5067-1511; Agilent Technologies, Inc.) on Bioanalyzer 2100 (cat. no. G2939BA; Agilent Technologies, Inc.). cDNA was synthesized using RT^2 First Strand kit (cat. no. 330404; Qiagen GmbH) with 0.5 μg total RNA, according to the manufacturer's protocol. cDNA was stored at -20˚C until quantitative PCR (qPCR) was performed.

qPCR. To quantify relative gene expression, ViiA7 Real-Time PCR System (Thermo Fisher Scientific, Inc.) was used. qPCR study design adhered to the Minimum Information for Publication of the guidelines for qPCR experiments (33). The reaction mixture contained 650 μl 2X RT^2 SYBR Green Mastermix (cat. no. 330502; Qiagen GmbH), 102 μl cDNA synthesis reaction (cDNA diluted with nuclease-free water according to the manufacturer's protocol) and 548 μl nuclease-free water. PCR components were dispensed into 384-well RT^2 lncRNA PCR Array (cat. no. 330721; Qiagen GmbH) according to the manufacturer's protocol. Each RT^2 lncRNA PCR Array Human Cancer PathwayFinder (cat. no. 330721; Qiagen GmbH; GeneGlobe ID: LAHS-002Z) included control elements for data normalization, detection of genomic DNA contamination, RNA sample quality and general PCR performance check (Table SI). Thermocycling conditions were as follows: Initial step at 95˚C for 10 min followed by 40 cycles of denaturation at 95˚C for 15 sec and annealing at 60˚C for 60 sec. The samples were analyzed in duplicate. The *ACTB*, *B2M*, *RPLP0*, *R7SK* and *SNORA73A* genes were used as reference genes for the normalization of results. For statistical analyses, the expression data were normalized and the $2^{-\Delta\Delta Cq}$ method was used to determine relative expression (34).

RNASeq. RNASeq was performed in 20 pairs of primary tumor and adjacent non-malignant renal tissue. Libraries were prepared using 0.5 μg total RNA with QuantSeq 3'mRNA-Seq Library Prep kit FWD and PCR Add-on kit for Illumina (cat. no. 015.96 and 020.96, respectively; both Lexogen GmbH) according to the manufacturer's protocol. Bioanalyzer 2100 and High Sensitivity DNA kit (cat. no. 5067-4626; Agilent Technologies, Inc.) were used for quality control of prepared libraries. Libraries were quantified by qPCR, KAPA Library Quantification kit Illumina® Platforms (cat. no. 07960140001; Fritz Hoffman-La Roche Ltd). The equimolar pool (4 nM) of prepared libraries was sequenced on NextSeq 500 platform (Illumina, Inc.) with NextSeq 500/550 High Output kit v2.5

Table I. Baseline clinical characteristics of patients with metastatic renal cell carcinoma.

Characteristic	lncRNA profiling (n=38)	mRNA profiling (n=20)
Median age at treatment initiation, years	64.5±8.8	64.5±8.5
Sex (%)		
Male	26 (68)	15 (75)
Female	12 (32)	5 (25)
Histopathological grade (%)		
G1	8 (21)	4 (20)
G2	12 (32)	6 (30)
G3	16 (42)	9 (45)
Not available	2 (5)	1 (5)
Stage at diagnosis (%)		
I	3 (8)	1 (5)
II	0 (0)	0 (0)
III	18 (47)	8 (40)
IV	17 (45)	11 (55)
Distant metastasis (%)		
Synchronous	16 (42)	11 (55)
Metachronous	22 (58)	9 (45)
MSKCC risk (%)		
Favorable	7 (18)	3 (15)
Intermediate	31 (82)	17 (85)
First-line objective response to sunitinib		
Complete response	6 (16)	5 (25)
Partial response	14 (37)	5 (25)
Progressive disease	18 (47)	10 (50)

Memorial Sloan-Kettering Cancer Center; lnc, long non-coding.

(1x75 bp, single read; cat. no. 20024906; Illumina, Inc.) in one run [seeding concentration 1.8 pM measured on Quibit 4.0 with dsDNA High Sensitivity Assay kit (cat. no. Q32851; Thermo Fisher Scientific, Inc.)]. RNASeq of all samples was performed in sufficient depth (~10 million reads/sample) for the detection of lowly expressed genes. Quality control of raw RNASeq data was performed with the FastQC v0.11.9 package (35).

Statistical analysis

lncRNA expression analysis. Statistical analysis of associations between lncRNA expression and clinical data of patients were performed by SPSS (v16.0; SPSS, Inc.) or GraphPad Prism (v6.0; Dotmatics). The distribution of most lncRNAs deviated from normality, and non-parametric statistical tests were used. Kruskal-Wallis test was used for evaluation of association between lncRNA expression profile and clinical parameters such as clinical stage, primary tumor size, histopathological grading and MSKCC risk. Mann-Whitney test was used for evaluation of associations between lncRNA expression profile and response to sunitinib, sex, presence of regional lymph node metastasis, type of distant metastatic spread (synchronous or metachronous) and comparison of lncRNA profile between primary tumor and paired non-neoplastic tissue. Spearman rank test was used for evaluating the correlation between the lncRNA expression levels and age of patients. Log-rank test and Kaplan-Meier plots were used to identify associations of lncRNA expression levels with PFS and OS in months. Patients were divided according to the median expression of a given lncRNA. PFS was defined as time between sunitinib treatment initiation and first documented progression or death or patient censoring. OS was defined as time from sunitinib treatment initiation until the date of death or patient censoring. All patients with OS >60 months were censored at this time point. Cox regression was performed to assess the hazard ratio (HR) with a 95% confidence interval (CI). Two-sided P-value was calculated for all statistical analyses. The false discovery rate (FDR) test was applied according to Benjamini and Hochberg (36) and Q-values were computed for each comparison. Q<0.05 was considered to indicate a statistically significant difference. The 'good responders group' was defined as patients who achieved CR or PR, while 'poor responders group' was defined as patients who achieved PD.

mRNA expression analysis. For gene annotation, Ensembl v101 (genome assembly GRCh38.p13) was used (37). A pseudoaligment approach for gene quantification by kallisto v0.46.1 was used (38). Differential expression analysis was carried out with the edgeR v3.42.2 package in R (39). Differentially expressed genes with log fold-change (FC)>2 and Q<0.001 (Benjamini Hochberg FDR correction) were considered statistically significant for comparison of tumor vs. non-malignant tissue, while for good vs. poor responders, Q<0.05 (Benjamini Hochberg FDR correction) was considered statistically significant. Expression data in the normalized format (transcripts per million) were used for analysis of associations between mRNA expression and clinical data of patients only for significantly differentially expressed protein-coding genes. The statistical tests used for these analyses were the same as those for lncRNAs.

Spearman correlation analysis was used to evaluate correlation between mRNA and lncRNA expression. R>0.8 and Q<0.001 [Bonferroni correction (40)] were considered to indicate a statisitcally significant difference. The complete set of 84 examined lncRNAs and 11,342 protein-coding transcripts with an interquartile range of expression values >0.1, with the exception of pseudogenes and uncharacterized proteins, were incorporated into the analysis.

Pathway annotation of protein-coding genes associated with lncRNAs was performed with the Reactome database (41). Q<0.05 (Benjamini Hochberg FDR correction) was considered to indicate a statistically significant difference.

Results

lncRNA profile between the primary tumor and paired non-neoplastic tissues. The levels of lncRNAs were detected by qPCR in 20 pairs of primary tumor and adjacent non-neoplastic tissue samples. Comparison of lncRNA expression profile revealed 50 differentially expressed lncRNAs (Table II). A significantly higher expression of 13 lncRNAs

Table II. Significant differences in expression of cancer-associated lncRNAs between primary tumor and adjacent non-malignant tissue (n=20 pairs).

lncRNA	Expression change	P-value	Q-value
ADAMTS9-AS2	Downregulated	0.011	0.021
AIRN	Downregulated	0.021	0.035
BANCR	Downregulated	<0.001	<0.001
BLACAT1	Downregulated	0.021	0.035
CAHM	Downregulated	<0.001	<0.001
CBR3-AS1	Downregulated	<0.001	<0.001
CDKN2B-AS1	Upregulated	<0.001	<0.001
CRNDE	Upregulated	0.003	0.006
DGCR5	Upregulated	<0.001	<0.001
EMX2OS	Downregulated	<0.001	<0.001
FTX	Downregulated	<0.001	<0.001
GACAT1	Downregulated	0.040	0.063[a]
GAS6-AS1	Upregulated	0.001	0.002
H19	Downregulated	0.006	0.012
HAND2-AS1	Downregulated	<0.001	<0.001
HEIH	Downregulated	0.046	0.072[a]
HIF1A-AS1	Downregulated	0.020	0.035
HIF1A-AS2	Upregulated	<0.001	<0.001
HNF1A-AS1	Downregulated	<0.001	<0.001
HOTAIRM1	Downregulated	<0.001	<0.001
HOXA11-AS	Downregulated	<0.001	<0.001
HOXA-AS2	Downregulated	<0.001	<0.001
HULC	Downregulated	<0.001	<0.001
IPW	Downregulated	<0.001	<0.001
KCNQ1OT1	Downregulated	<0.001	<0.001
KRAASP1	Downregulated	0.002	0.035
LINC00152	Upregulated	0.001	0.002
LINC00887	Upregulated	<0.001	<0.001
LINC00963	Downregulated	<0.001	<0.001
LINC01233	Downregulated	<0.001	<0.001
LINC01234	Upregulated	0.007	0.014
LUCAT1	Upregulated	<0.001	<0.001
MALAT1	Downregulated	0.008	0.015
MEG3	Downregulated	<0.001	<0.001
MIR155HG	Downregulated	0.040	0.063[a]
MIR17HG	Upregulated	<0.001	<0.001
MIR31HG	Downregulated	0.001	0.002
MRPL23-AS1	Downregulated	0.001	0.002
NAMA	Downregulated	0.002	0.004
NBR2	Downregulated	<0.001	<0.001
NEAT1	Downregulated	<0.001	<0.001
NRON	Downregulated	0.013	0.024
POU5F1P5	Downregulated	0.038	0.063[a]
PRNCR1	Downregulated	<0.001	<0.001
PTCSC3	Downregulated	<0.001	<0.001
PVT1	Upregulated	<0.001	<0.001
RMRP	Downregulated	0.006	0.012
RMST	Downregulated	<0.001	<0.001
SNHG16	Upregulated	0.006	0.012
SUMO1P3	Downregulated	0.001	0.002
TUG1	Downregulated	<0.001	<0.001
WT1-AS	Downregulated	<0.001	<0.001
ZFAS1	Upregulated	0.002	0.004

[a]Non-significant. lncRNA, long non-coding RNA.

in carcinoma compared with paired non-neoplastic tissue was observed. By contrast, the levels of 37 lncRNAs were significantly decreased in carcinoma. In addition, four differentially expressed lncRNAs (GACAT1, HEIH, MIR155HG and POU5F1P5) passed the P-value cut-off (P<0.05) but not the FDR correction (Benjamini and Hochberg) (Table II).

Associations of lncRNA expression profile with baseline clinical data. The levels of the lncRNAs in tumors from 38 patients were evaluated for their associations with the clinical data. The association of lncRNA expression profile with sex, grade, MSKCC risk, primary tumor size and synchronous or metachronous distant metastatic spread is shown in Table SII. However, only higher lncRNA TSIX and XIST levels in females were significant after FDR correction.

Associations of lncRNA expression profile with objective response and survival. The expression of 10 lncRNAs (ADAMTS9-AS2, CDKN2B-AS1, CRNDE, EMX2OS, HEIH, HNF1A-AS1, IPW, LINC00963, NRON and PTENP1) was upregulated, while the expression of LINC00261, LINC01234, TUG1 and TUSC7 was downregulated in good compared with poor responders (Table III). The downregulation of TUSC7 lncRNA, which remained significant after FDR correction, was the most important finding.

Association of lncRNA expression profile with PFS and OS showed that patients with expression levels of HNF1A-AS1 (HR=0.193; 95% CI=0.051-0.724) and IPW (HR=0.18; 95% CI=0.03-0.95) above the median (high expression) had prolonged PFS compared with those with levels below the median (Fig. 1A and B). Expression levels of TUSC7 above the median (high expression) were associated with poor PFS (HR=4.3; 95% CI=1.35-13.70; Fig. 1C) and OS (HR=4.15; 95% CI=1.38-12.48; Fig. 1D).

mRNA transcriptome profile between primary tumor and adjacent non-neoplastic tissues. Analysis of differential expression between primary tumors and paired adjacent non-neoplastic tissues was performed in 20 patients, whose baseline clinical data are summarized in Table I. In total, 768 significantly differentially expressed genes were identified. A total of 462 and 306 genes were down- and upregulated, respectively, in tumor compared with non-neoplastic tissue (Table SIII). The volcano plot and top 10 down- and upregulated genes are shown in Fig. 2A and B.

Association of mRNA expression profile with objective response and survival. Among all protein-coding genes, only one significant association with objective response to sunitinib

Table III. Association of expression levels of cancer-associated lncRNAs with the objective response to sunitinib in good (n=20) vs. poor (n=18) responders.

lncRNA	Expression change	P-value	Q-value
ADAMTS9-AS2	Upregulated	0.026	0.196[a]
CDKN2B-AS1	Upregulated	0.019	0.196[a]
CRNDE	Upregulated	0.011	0.154[a]
EMX2OS	Upregulated	0.024	0.196[a]
HEIH	Upregulated	0.047	0.288[a]
HNF1A-AS1	Upregulated	0.028	0.196[a]
IPW	Upregulated	0.002	0.084[a]
LINC00261	Downregulated	0.010	0.154[a]
LINC00963	Upregulated	0.024	0.196[a]
LINC01234	Downregulated	0.011	0.154[a]
NRON	Upregulated	0.024	0.196[a]
PTENP1	Upregulated	0.011	0.154[a]
TUG1	Downregulated	0.033	0.213[a]
TUSC7	Downregulated	<0.001	<0.001

[a]Non-significant. lncRNA, long non-coding RNA.

was identified: Significantly upregulated *CLIP4* expression was observed in primary tumor tissues of poor responders compared with good responders (logFC=-1.9; Q=0.02; Fig. 3).

mRNA-lncRNA co-expression networks. The complete mRNA expression profile was compared with 84 cancer-associated lncRNA in 20 primary tumor tissues. This revealed 107 significant associations (R>0.8 and Q<0.001). In total, levels of 26 lncRNAs were significantly correlated with mRNA expression levels of 65 protein-coding genes (Fig. 4). The lncRNAs with the highest number of correlations with protein-coding genes were MEG3 (14 positive correlations) and SNHG16 (7 negative correlations; Fig. 4; Table SIV). MEG3 was positively correlated with expression of *ABHD2*, *BPIFA3*, *CARD8*, *CBS*, *CDK12*, *IPO4*, *MB*, *OSER1*, *POLR3F*, *RNF126*, *SLC6A1*, *SPHK2*, *TRIM60* and *UNC45A* (Table SV). SNHG16 was negatively correlated with the expression of *ABCF3*, *AP2M1*, *CLN8*, *DNMT3A*, *IQCB1*, *PDE4D* and *SSBP4*.

Among the deregulated lncRNAs in poor responders to sunitinib, lncRNA ADAMTS9-AS2 was positively correlated with expression of *NKX2-1* and *INTS8* mRNAs (R=0.83 and R=0.84, respectively). By contrast, CRNDE lncRNA displayed only negative correlations, namely with the expression of *DPH5* and *CDK11B* mRNAs (R=-0.84 and R=-0.84, respectively). The expression of lncRNA EMX2OS was positively correlated with that of *RHOBTB3* and *UGT2A1* and negatively correlated with that of *ITGA2* mRNA (R=0.84, R=0.85 and R=-0.87, respectively), while LINC01234 was positively correlated with the expression of *TUBB4A* mRNA (R=0.85).

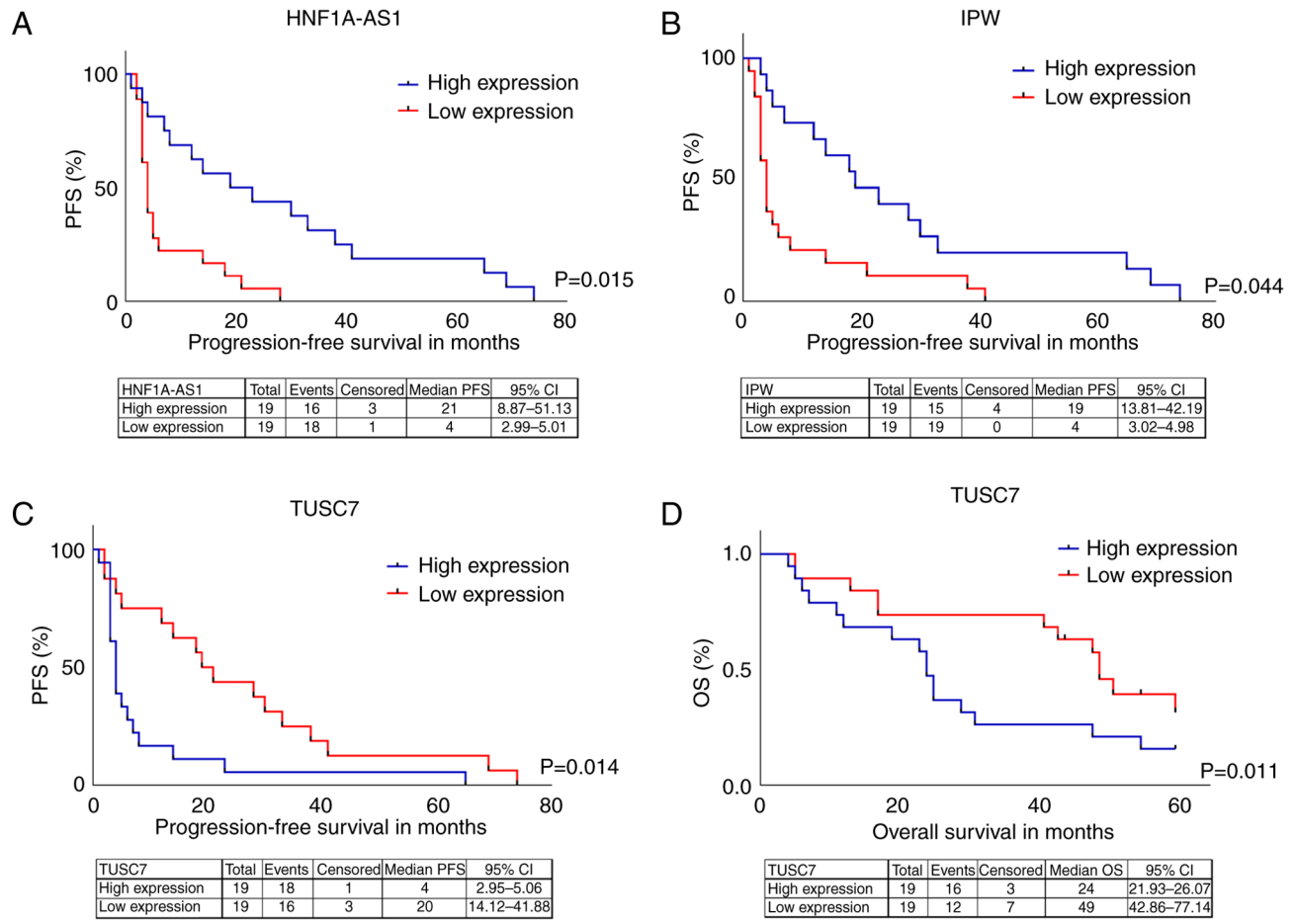
Pathway analysis was performed with the Reactome database for the aforementioned lncRNAs (Fig. 5). Genes correlated with the lncRNA MEG3 were significantly enriched in metabolism (*SPHK2* and *CBS*), immune system/response (*CARD8* and *POLR3F*), apoptosis (*CARD8*), gene expression (*POLR3F* and *CDK12*), neuronal system (*SLC6A1*) and transport (*MB*) pathways. The function of other genes correlated with MEG3 (*BPIFA3, OSER1, ABHD2, UNC45A, TRIM60* and *IPO4*) has not been identified to date (Fig. 5A). SNHG16 lncRNA-correlated genes were significantly enriched in GTPase cycle (*CLN8*), signaling (*PDE4D* and *AP2M1*), DNA methylation (*DNMT3A*) and plasma membrane (*IQCB1*), and two genes had unknown function (*SSBP4* and *ABCF3*; Fig. 5B). Genes correlating with ADAMTS9-AS2 lncRNA were significantly enriched in gene expression (*INTS8*) and surfactant metabolism (*NKX2-1*; Fig. 5C). *MUCL3* gene correlating with CDKN2B-AS1 lncRNA has an unknown function (Fig. 5D). *TUBB4A* correlating with LINC01234 lncRNA was significantly enriched in protein transport and folding (Fig. 5E). Genes correlating with CRNDE lncRNA were significantly enriched in metabolic pathways (*DPH5*) and cell cycle regulation (*CDK11B*; Fig. 5F), while genes correlating with lncRNA EMX2OS were enriched in drug metabolism (*UGT2A1*), ATPase cycle (*RHOBTB3*) and signaling pathways/cell interactions (*ITGA2*; Fig. 5G). Results are summarized in Table SVI.

Discussion

lncRNAs play a complex role in cancer biology (9). Although the value of lncRNAs as potential prognostic or predictive biomarker in patients with mRCC has already been suggested (16), associations between lncRNA profile and outcome focused on specific types of systemic targeted therapy remain underexplored.

The present study analyzed associations between the expression profile of cancer-specific lncRNAs selected using Human Cancer PathwayFinder and the outcome of patients with mRCC treated with sunitinib as first-line therapy. The results suggested a potential prognostic and/or predictive role of HNF1A-AS1, IPW and TUSC7 among 84 cancer-specific lncRNAs. Moreover, full transcriptome analysis protein-coding genes was performed; *CLIP4* was associated with objective response. Furthermore, MEG3 and SNHG16 lncRNAs were not only dysregulated in mRCC, but also strongly associated with the expression levels of several protein-coding genes, suggesting a complex functional significance and potential use in targeted therapies.

Thus, according to the present study, downregulated expression of the TUSC7 lncRNA may serve as a negative prognostic and predictive biomarker candidate in follow-up studies on mRCC and other malignancies. It was downregulated in tumors compared with non-malignant renal tissue and upregulated in tumors of poor responders and patients with worse survival (both PFS and OS). To the best of our knowledge, TUSC7 has not been previously reported in connection with renal malignancies. In non-malignant tissues, TUSC7 expression is upregulated in testes (42). TUSC7-regulated cellular processes play a tumor-suppressor function in various types of cancer, for example, inhibiting the proliferation rate and migration of tumor cells in epithelial-mesenchymal transition (EMT) in colorectal cancer cell lines and tissue (43) or osteosarcoma cells (44). According to a previous study, TUSC7 downregulation is an independent biomarker of poor prognosis in patients with triple-negative breast cancer (45),



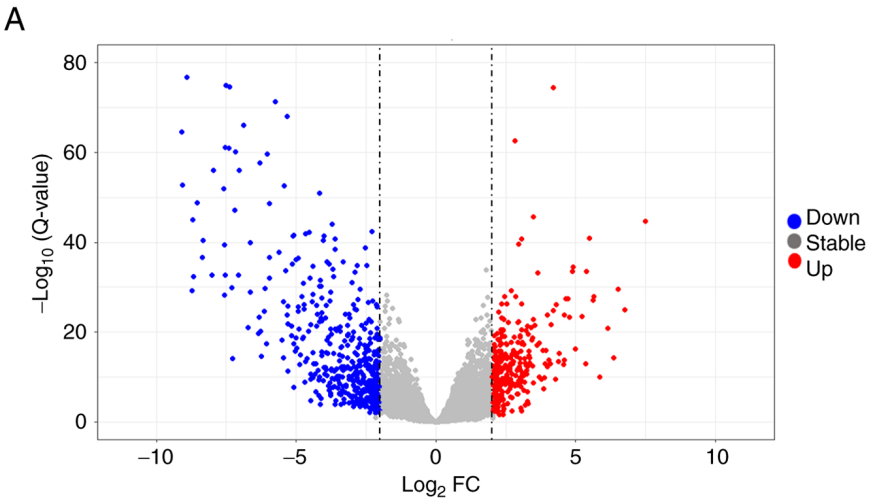
HNF1A-AS1	Total	Events	Censored	Median PFS	95% CI
High expression	19	16	3	21	8.87–51.13
Low expression	19	18	1	4	2.99–5.01

IPW	Total	Events	Censored	Median PFS	95% CI
High expression	19	15	4	19	13.81–42.19
Low expression	19	19	0	4	3.02–4.98

TUSC7	Total	Events	Censored	Median PFS	95% CI
High expression	19	18	1	4	2.95–5.06
Low expression	19	16	3	20	14.12–41.88

TUSC7	Total	Events	Censored	Median OS	95% CI
High expression	19	16	3	24	21.93–26.07
Low expression	19	12	7	49	42.86–77.14

Figure 1. Association of expression of lncRNA with PFS. Association of (A) HNF1-AS1, (B) IPW and (C) TUSC7 with PFS. (D) Association of TUSC7 with OS. lncRNA, long non-coding RNA; OS, overall survival; PFS, progression-free survival; CI, confidence interval; HNF1-AS1, hepatic nuclear factor 1 homeobox A antisense RNA; IPW, imprinted in Prader-Willi syndrome; TUSC7, tumor suppressor candidate 7.



B

Gene	LogFC	Q-value
Top 10 upregulated genes		
BHLHE41	4.19	3.18×10^{-75}
CAV2	2.82	2.54×10^{-63}
SCARB1	3.47	1.79×10^{-46}
FABP6	7.49	1.70×10^{-45}
CDKN2A	5.49	1.40×10^{-41}
PFKP	3.06	2.14×10^{-41}
CAV1	2.97	2.25×10^{-40}
EGLN3	4.92	2.72×10^{-35}
HILPDA	5.83	2.76×10^{-34}
NNMT	4.87	3.28×10^{-34}
Top 10 downregulated genes		
ATP6V0A4	−8.85	1.78×10^{-161}
AQP2	−11.81	2.00×10^{-126}
FGF9	−7.84	2.55×10^{-119}
TFAP2B	−8.08	2.87×10^{-101}
SOST	−7.32	1.83×10^{-99}
SLC12A3	−10.21	5.11×10^{-99}
RHCG	−9.59	2.18×10^{-90}
UMOD	−12.97	2.20×10^{-88}
CA10	−7.17	2.39×10^{-84}
TMEM213	−6.33	4.86×10^{-83}

Figure 2. Differential expression analysis of protein-coding genes between primary tumor and adjacent non-malignant tissue samples. (A) Volcano plot of the most significant results from differential expression analysis (logFC>2; Q<0.001). (B) Top 10 most up- and downregulated genes (n=20).

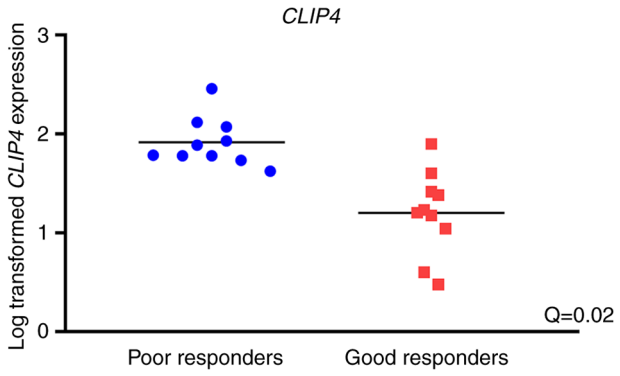


Figure 3. Significantly higher *CLIP4* expression is observed in poor vs. good responders (n=20) as shown in a scatter plot where the mean is indicated by a horizontal line. *CLIP4*, CAP-Gly domain containing linker protein family member 4.

which contradicts the observations of the present study; this is probably due to the different nature of tumors of individual tissue types. TUSC7 is regulated by p53 *in vitro* (45) and thus, the functional status of the p53 pathway may affect the TUSC7 prognostic significance. Patients with triple-negative breast cancer have a high prevalence (~75%) of TP53 mutation (46). Thus, TUSC7 may have different tissue-specific functions reflecting the p53 status in a specific type and histological subtype of carcinoma, which may partly explain discordant results.

HNF1A-AS1 and IPW served as positive predictive biomarkers in the present study as their upregulation in tumors was associated with good response and prolonged PFS. HNF1A-AS1 lncRNA is upregulated mainly in gastrointestinal, liver and kidney tissues (47). HNF1A-AS1 expression is often deregulated in cancer and it serves roles in cell proliferation, invasion, migration and apoptosis primarily via cooperation with microRNAs (miRs) or by regulating the EMT process (48-51). HNF1A-AS1 serves as a tumor promoter, but also as a tumor suppressor, as shown by Zhang *et al* (51). Upregulation of HNF1A-AS1 is demonstrated in numerous tumors, such as osteosarcoma, gastrointestinal, breast, lung or cervical carcinoma, while it is downregulated in gastroenteropancreatic and neuroendocrine neoplasm and oral squamous cell carcinoma (48,51). In the present study, HNF1A-AS1 was significantly downregulated in patients with mRCC. In connection with development of tumors and tumor progression, HNF1A-AS1 promotes lung cancer cell proliferation and invasion via regulating miR-17-5p (49). Expression of miR-149-5p negatively correlated with HNF1A-AS1 in tissue of patients with non-small cell lung cancer (NSCLC) and in NSCLC cell lines (50). In a meta-analysis focusing on the usefulness of HNF1A-AS1 as a prognostic marker in malignant tumor, high HNF1A-AS1 expression correlated with poor OS and disease-free survival in patients with colorectal, bladder and lung cancer and osteosarcoma (52). On the other hand, HNF1A-AS1 serves as a tumor suppressor in other studies (53,54), in accordance with the present study. Dang *et al* (55) showed that downregulation of HNF1A-AS1 in gastric cancer is associated with tumor size and concentration of the protein serum biomarkers carcinoembryonic antigen and carbohydrate antigen 19-9, as well as with the protein expression of ribonucleotide reductase subunit M1 in tissue samples. In liver cancer, HNF1A-AS1 is downregulated, and could inhibit the proliferative and metastatic abilities of hepatocellular carcinoma xenograft tumors (54). Thus, the function and mechanism of action of HNF1A-AS1 depends on cell specificity and tumor type.

IPW is a nuclear lncRNA with tissue-specific expression. It has been shown to regulate genomic imprinting, a subject for a study of transcriptional and post-transcriptional-based gene regulation (56). The highest levels of IPW are identified in the nervous system, based on estimation by BioGPS microarray (57). IPW forms part of a six-lncRNA prognostic signature in gastric cancer (58) and recently it was reported to be downregulated in head and neck squamous cell cancer (HNSCC) cells in comparison with normal keratinocyte cells *in vitro* (59). In addition, downregulation of expression of IPW is associated with worse OS in patients with HNSCC (59). The present study found an association between downregulation of IPW expression and poor objective response and worse PFS in patients with mRCC, but not with OS, suggesting predictive, rather than prognostic, value. To the best of our knowledge, the role of IPW in ccRCC has not been investigated to date.

To address the complexity of lncRNA-mRNA interacting networks, the present study complemented the targeted lncRNA analyses with assessment of the coding transcriptome in a subset of patients with mRCC. The analysis revealed upregulation of *CLIP4* in patients with mRCC with poor response to sunitinib. *CLIP4* encodes the intracellular CAP-Gly domain containing linker protein family member 4, a protein involved in cytoplasmic microtubule organization (Gene Ontology:0031122) (60). Park *et al* (61) analyzed the transcriptome of patients with early-stage ccRCC (n=24) using RNASeq and subsequently suggested and validated the association of *CLIP4* upregulation with poor prognosis. In addition, *CLIP4* mutations are enriched 3-fold in patients with aggressive ccRCC defined as tumors exhibiting synchronous metastasis, early recurrence or cancer-specific mortality, compared with patients without aggressive ccRCC (61). Ahn *et al* (62) noted that upregulation of *CLIP4* expression was associated with synchronous metastasis in ccRCC, and an *in vitro* functional study showed that *CLIP4* significantly increases cell migration and viability in ccRCC (62). Taken together, several studies, including the current one, suggest *CLIP4* upregulation as a poor prognosis biomarker in patients with ccRCC.

In the present study, two lncRNAs (MEG3 and SNHG16) were significantly associated with individual gene expression profile of tumors from patients with mRCC. MEG3 expression was positively correlated with expression of 14 protein-coding genes, and pathway enrichment analysis suggested an involvement of genes from biological processes such as cell metabolism, apoptosis, transport and immune system regulation. A network involving MEG3 lncRNA may serve a role in prognosis and therapy response. Gong *et al* (63) revealed a positive correlation of ST3 β-galactoside α-2,3-sialyltransferase 1 (ST3Gal1) expression with MEG3 in ccRCC, and suggested a potential role of the MEG3/ST3Gal1/epidermal growth factor receptor

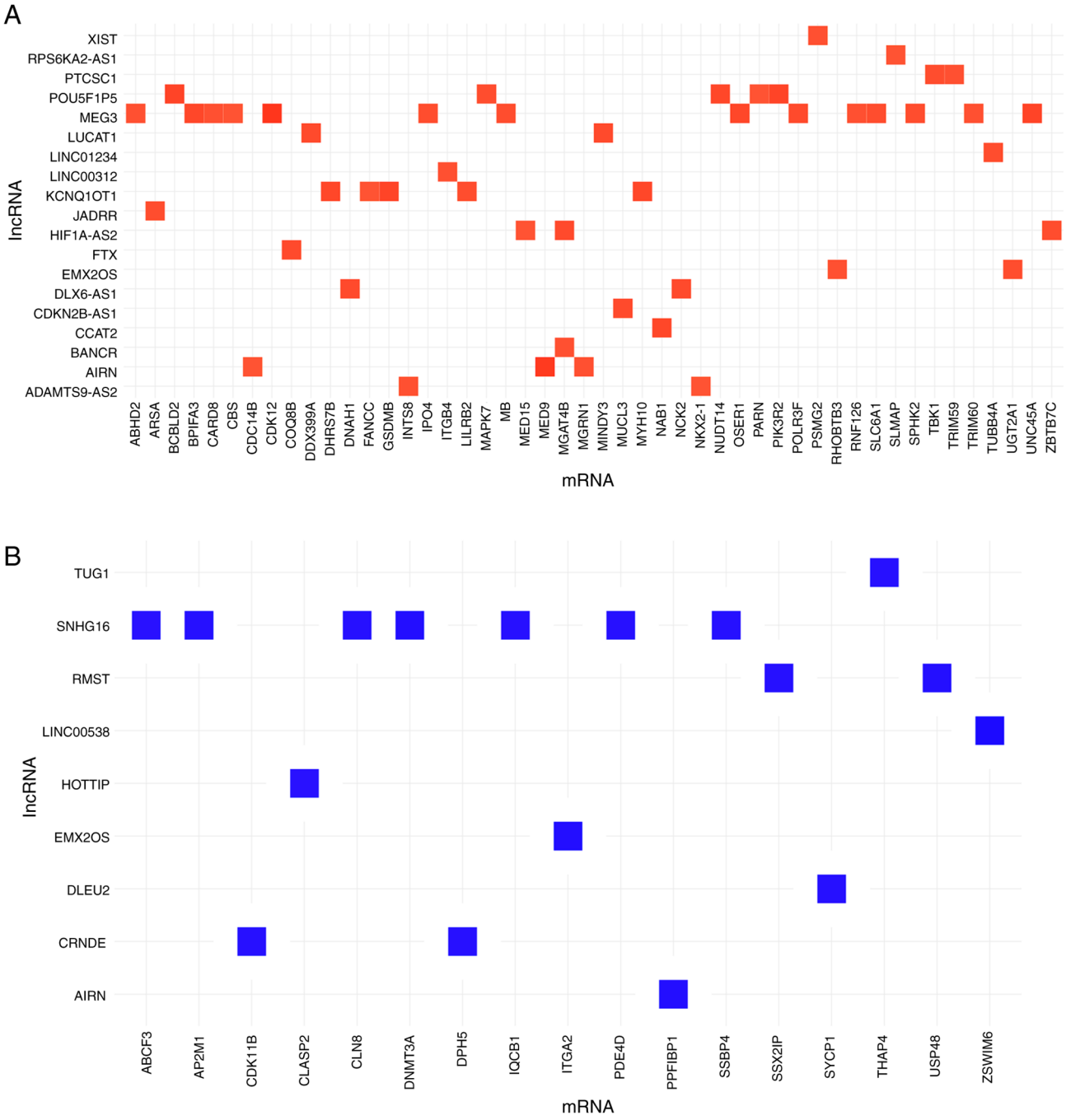


Figure 4. Significant correlations between expression of protein-coding genes and lncRNAs in primary tumor tissue. (A) Positive and (B) negative correlations. Only results with Spearman correlation coefficient R>0.8 and Q<0.001 are shown. lnc, long non-coding.

axis in ccRCC progression. Upregulation of MEG3 induces apoptosis via the reduction of Bcl-2 and procaspase-9 protein and the promotion of cytochrome c release into the cytoplasm (14). The present study found a significant downregulation of MEG3 in ccRCC, confirming the results of previous studies (14,63,64) reporting downregulation of MEG3 in tumors of patients with ccRCC and ccRCC cell lines compared with non-malignant renal tissues.

SNHG16 lncRNA was upregulated in tumors and negatively correlated with the expression of seven protein-coding genes in the present study. Functionally, SNHG16 promotes cell proliferation and suppresses apoptosis via interaction with miR-1301-3p, leading to the upregulation of *STAR* expression in ccRCC cells (65). In agreement with a previous study (65), the present study confirmed that SNHG16 may serve a role as an oncogene in ccRCC.

There are limitations to the present study, including a small sample size and a retrospective design. Nevertheless, the current study focused on the metastatic stage of ccRCC, which is not as common as the early stages of ccRCC and there are limited options to obtain fresh frozen tissue samples from patients with mRCC, particularly those with synchronous metastatic disease. The next limitation is that qPCR for lncRNA profile measures only the expression of a limited number of pre-selected lncRNAs. Another limitation is that sunitinib monotherapy is no longer the first choice of

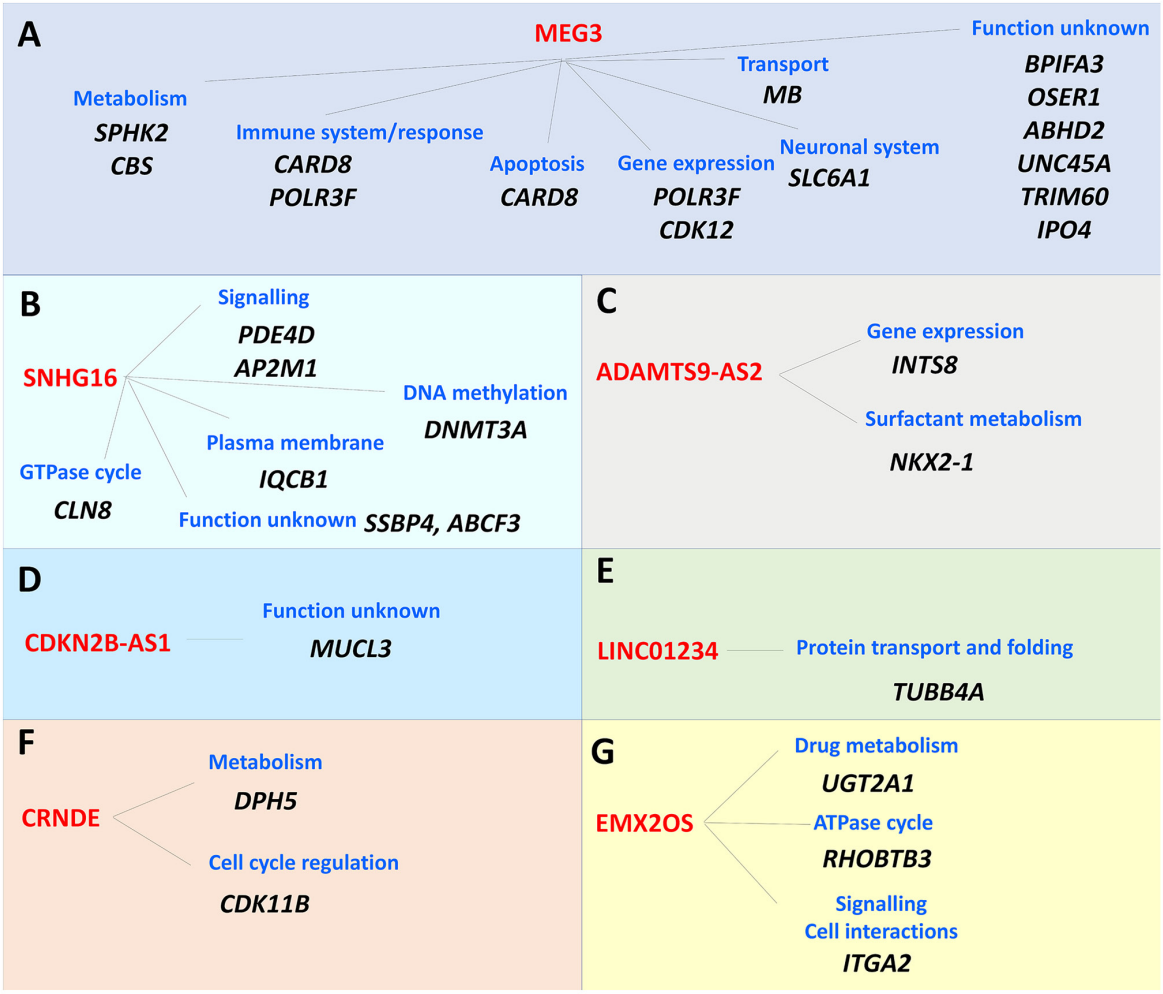


Figure 5. Pathway enrichment analysis using Reactome database for selected lncRNA-mRNA correlations. Genes correlated with (A) MEG3, (B) SNHG16, (C) ADAMTS9-AS2, (D) CDKN2B-AS1, (E) LINC01234, (F) CRNDE and (G) EMX2OS lncRNAs are presented. lncRNA, long non-coding RNA.

first-line treatment and it has been replaced with immunotherapy combination regimens, represented by combinations of TKI plus ICI or ICI plus ICI. Finally, the lack of functional studies of the identified candidate lncRNA biomarkers is another limitation.

On the other hand, TKIs are still widely used in the treatment of mRCC, and a search for candidate predictive biomarkers for these agents could bring progress in the personalized use of TKIs in monotherapy, even in combination with immunotherapy (7,8). Moreover, the followed-up group of patients with mRCC was clinically well-characterized, particulary during first-line systemic treatment and represented a uniquely homogenous group of patients with mRCC, coupled with the prospectively updated outcome data. Furthermore, high-throughput RNASeq methodology was used for estimation of the whole coding transcriptome in 20 patients with mRCC, and the data of the current study may serve as a hypothesis-generating screening for larger functional and replication studies in independent cohorts of patients with mRCC to confirm the observations of the present study. Functional studies of the candidate lncRNA biomarkers identified in the present study are ongoing.

In conclusion, the present study provided novel information within the lncRNA field and their clinical role as molecular biomarkers of therapeutic response in patients with mRCC. Among 84 cancer-associated lncRNAs, HNF1A-AS1, IPW and TUSC7 dysregulation was associated with outcome of patients with mRCC treated with sunitinib. Moreover, the predictive association was revealed for the *CLIP4* protein-coding transcript. Additionally, significant associations of MEG3 and SNHG16 with several protein-coding transcripts, creating complex interactive networks, were identified and confirmed by *in silico* predictions of molecular and biological function. The aforementioned molecules represent putative candidates for predictive and prognostic biomarkers in precision and personalized therapy of mRCC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. RNASeq data are available in the National Center for Biotechnology Information database's Sequence Read Archive repository, BioProject no. PRJNA932699 (ncbi.nlm.nih.gov/bioproject/PRJNA932699/).

Authors' contributions

RV, OF, PS and TT conceptualized the study. TT, KK and KS performed experiments. TT and KS performed data analysis. MH, OH and KP provided and characterized tissue resources, and described all clinical parameters and characteristics included in this paper. KS and TT visualized the data. TT, KK, KS and OF wrote the manuscript. OF, RV and PS reviewed and edited the manuscript. PS supervised the study and created essential parts of the discussion section. TT and KS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

The present study involving human subjects was conducted according to the guidelines of the Declaration of Helsinki and approved by The Ethics Committee of the Faculty of Medicine and University Hospital Pilsen, Charles University (approval no. 302/2020). Written informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Not applicable.

Competing interests

Ondrej Fiala received payment or honoraria for lectures, presentations, speakers' bureaus, or educational events from Roche, Janssen, GSK, MSD, Pierre Fabre, BMS and Pfizer unrelated to this project.

References

1. Znaor A, Lortet-Tieulent J, Laversanne M, Jemal A and Bray F: International variations and trends in renal cell carcinoma incidence and mortality. Eur Urol 67: 519-530, 2015.
2. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, Verweij J, Van Glabbeke M, van Oosterom AT, Christian MC and Gwyther SG: New Guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 92: 205-216, 2000.
3. National Cancer Institute (NCI): Clear Cell Renal Cell Carcinoma-NCI. https://www.cancer.gov/pediatric-adult-rare-tumor/rare-tumors/rare-kidney-tumors/clear-cell-renal-cell-carcinoma. Accessed February 1, 2023.
4. Xue J, Chen W, Xu W, Xu Z, Li X, Qi F and Wang Z: Patterns of distant metastases in patients with clear cell renal cell carcinoma - - A population-based analysis. Cancer Med 10: 173-187, 2020.
5. Dabestani S, Thorstenson A, Lindblad P, Harmenberg U, Ljungberg B and Lundstam S: Renal cell carcinoma recurrences and metastases in primary non-metastatic patients: A population-based study. World J Urol 34: 1081-1086, 2016.
6. Eggers H, Schünemann C, Grünwald V, Rudolph L, Tiemann ML, Reuter C, Anders-Meyn MF, Ganser A and Ivanyi P: Improving survival in metastatic renal cell carcinoma (MRCC) patients: Do elderly patients benefit from expanded targeted therapeutic options? World J Urol 40: 2489-2497, 2022.
7. Sheng IY and Ornstein MC: Ipilimumab and nivolumab as first-line treatment of patients with renal cell carcinoma: The evidence to date. Cancer Manag Res 12: 4871-4881, 2020.
8. Motzer RJ, Jonasch E, Agarwal N, Alva A, Baine M, Beckermann K, Carlo MI, Choueiri TK, Costello BA, Derweesh IH, *et al*: Kidney cancer, Version 3.2022, NCCN clinical practice guidelines in oncology. J Natl Compr Canc Netw 20: 71-90, 2022.
9. Statello L, Guo CJ, Chen LL and Huarte M: Gene regulation by long Non-Coding RNAs and Its biological functions. Nat Rev Mol Cell Biol 22: 96-118, 2021.
10. Shao K, Shi T, Yang Y, Wang X, Xu D and Zhou P: Highly expressed LncRNA CRNDE promotes cell proliferation through Wnt/β-Catenin signaling in renal cell carcinoma. Tumour Biol: Oct 6, 2016 (Epub ahead of print).
11. Wang L, Cai Y, Zhao X, Jia X, Zhang J, Liu J, Zhen H, Wang T, Tang X, Liu Y and Wang J: Down-regulated long non-coding RNA H19 inhibits carcinogenesis of renal cell carcinoma. Neoplasma 62: 412-418, 2015.
12. Wu Y, Liu J, Zheng Y, You L, Kuang D and Liu T: Suppressed expression of long Non-coding RNA HOTAIR inhibits proliferation and tumourigenicity of renal carcinoma cells. Tumour Biol 35: 11887-11894, 2014.
13. Zhang M, Lu W, Huang Y, Shi J, Wu X, Zhang X, Jiang R, Cai Z and Wu S: Downregulation of the long noncoding RNA TUG1 inhibits the proliferation, migration, invasion and promotes apoptosis of renal cell carcinoma. J Mol Histol 47: 421-428, 2016.
14. Wang M, Huang T, Luo G, Huang C, Xiao XY, Wang L, Jiang GS and Zeng FQ: Long Non-Coding RNA MEG3 induces renal cell carcinoma cells apoptosis by activating the mitochondrial pathway. J Huazhong Univ Sci Technolog Med Sci 35: 541-545, 2015.
15. Qiao HP, Gao WS, Huo JX and Yang ZS: Long Non-Coding RNA GAS5 functions as a tumor suppressor in renal cell carcinoma. Asian Pac J Cancer Prev 14: 1077-1082, 2013.
16. Li M, Wang Y, Cheng L, Niu W, Zhao G, Raju JK, Huo J, Wu B, Yin B, Song Y and Bu R: Long Non-Coding RNAs in renal cell carcinoma: A systematic review and clinical implications. Oncotarget 8: 48424-48435, 2017.
17. Zhang H, Yang F, Chen SJ, Che J and Zheng J: Upregulation of Long Non-Coding RNA MALAT1 correlates with tumor progression and poor prognosis in clear cell renal cell carcinoma. Tumour Biol 36: 2947-2955, 2015.
18. Posa I, Carvalho S, Tavares J and Grosso AR: A Pan-cancer analysis of MYC-PVT1 Reveals CNV-Unmediated deregulation and poor prognosis in renal carcinoma. Oncotarget 7: 47033-47041, 2016.
19. Wang Y, Liu J, Bai H, Dang Y, Lv P and Wu S: Long Intergenic Non-Coding RNA 00152 promotes renal cell carcinoma progression by epigenetically suppressing P16 and negatively regulates MiR-205. Am J Cancer Res 7: 312-322, 2017.
20. Xiao H, Bao L, Xiao W, Ruan H, Song Z, Qu Y, Chen K, Zhang X and Yang H: Long Non-Coding RNA Lucat1 is a poor prognostic factor and demonstrates malignant biological behavior in clear cell renal cell carcinoma. Oncotarget 8: 113622-113634, 2017.
21. Song EL, Xing L, Wang L, Song WT, Li DB, Wang Y, Gu YW, Liu MM, Ni WJ, Zhang P, *et al*: LncRNA ADAMTS9-AS2 inhibits cell proliferation and decreases chemoresistance in clear cell renal cell carcinoma via the MiR-27a-3p/FOXO1 axis. Aging (Albany NY) 11: 5705-5725, 2019.
22. Qu L, Ding J, Chen C, Wu ZJ, Liu B, Gao Y, Chen W, Liu F, Sun W, Li XF, *et al*: Exosome-Transmitted LncARSR promotes sunitinib resistance in renal cancer by acting as a competing endogenous RNA. Cancer Cell 29: 653-668, 2016.
23. Zhai W, Sun Y, Guo C, Hu G, Wang M, Zheng J, Lin W, Huang Q, Li G, Zheng J and Chang C: LncRNA-SARCC suppresses renal cell carcinoma (RCC) progression via altering the androgen receptor(AR)/MiRNA-143-3p signals. Cell Death Differ 24: 1502-1517, 2017.

24. Saad OA, Li WT, Krishnan AR, Nguyen GC, Lopez JP, McKay RR, Wang-Rodriguez J and Ongkeko WM: The Renal clear cell carcinoma immune landscape. Neoplasia 24: 145-154, 2022.
25. Roldán FL, Izquierdo L, Ingelmo-Torres M, Lozano JJ, Carrasco R, Cuñado A, Reig O, Mengual L and Alcaraz A: Prognostic gene expression-based signature in clear-cell renal cell carcinoma. Cancers (Basel) 14: 3754, 2022.
26. Flaifel A, Xie W, Braun DA, Ficial M, Bakouny Z, Nassar AH, Jennings RB, Escudier B, George DJ, Motzer RJ, *et al*: PD-L1 expression and clinical outcomes to cabozantinib, everolimus and sunitinib in patients with metastatic renal cell carcinoma: Analysis of the randomized clinical trials METEOR and CABOSUN. Clin Cancer Res 25: 6080-6088, 2019.
27. Qu Y, Lin Z, Qi Y, Qi Y, Chen Y, Zhou Q, Zeng H, Liu Z, Wang Z, Wang J, *et al*: PAK1 expression determines poor prognosis and immune evasion in metastatic renal cell carcinoma patients. Urol Oncol 38: 293-304, 2020.
28. Motzer RJ, Bacik J, Murphy BA, Russo P and Mazumdar M: Interferon-Alfa as a comparative treatment for clinical trials of new therapies against advanced renal cell carcinoma. J Clin Oncol 20: 289-296, 2002.
29. Fiala O, Finek J, Poprach A, Melichar B, Kopecký J, Zemanova M, Kopeckova K, Mlcoch T, Dolezal T, Capkova L and Buchler T: Outcomes according to MSKCC risk score with focus on the Intermediate-Risk Group in metastatic renal cell carcinoma patients treated with first-line sunitinib: A Retrospective analysis of 2390 Patients. Cancers (Basel) 12: 808, 2020.
30. European Medicines Agency (EMA): Sutent. European Medicines Agency, Amsterdam, 2021. https://www.ema.europa.eu/en/medicines/human/EPAR/sutent. Accessed May 23, 2023.
31. Eisenhauer EA, Therasse P, Bogaerts J, Schwartz LH, Sargent D, Ford R, Dancey J, Arbuck S, Gwyther S, Mooney M, *et al*: New response evaluation criteria in solid tumours: Revised RECIST guideline (Version 1.1). Eur J Cancer 45: 228-247, 2009.
32. Schroeder A, Mueller O, Stocker S, Salowsky R, Leiber M, Gassmann M, Lightfoot S, Menzel W, Granzow M and Ragg T: The RIN: An RNA integrity number for assigning integrity values to RNA measurements. BMC Mol Biol 7: 3, 2006.
33. Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, *et al*: The MIQE guidelines: Minimum information for publication of quantitative real-Time PCR Experiments. Clin Chem 55: 611-622, 2009.
34. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
35. Babraham Bioinformatics: FastQC: A Quality Control Tool for High Throughput Sequence Data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/. Accessed November 2, 2021.
36. Benjamini Y and Hochberg Y: Controlling the false discovery rate: A practical and powerful approach to multiple testing. J Royal Statistical Soc Series B (Methodological) 57: 289-300, 1995.
37. Ensembl: Human (GRCh38.p13). http://www.ensembl.org/Homo_sapiens/Info/Index. Accessed May 25, 2023.
38. Bray NL, Pimentel H, Melsted P and Pachter L: Near-optimal probabilistic RNA-Seq quantification. Nat Biotechnol 34: 525-527, 2016.
39. McCarthy DJ, Chen Y and Smyth GK: Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. Nucleic Acids Res 40: 4288-4297, 2012.
40. Bonferroni CE: Il Calcolo Delle Assicurazioni Su Gruppi Di Teste. Studi in onore del Professore Salvatore Ortu Carboni, 1935.
41. Gillespie M, Jassal B, Stephan R, Milacic M, Rothfels K, Senff-Ribeiro A, Griss J, Sevilla C, Matthews L, Gong C, *et al*: The reactome pathway knowledgebase 2022. Nucleic Acids Res 50: D687-D692, 2022.
42. GTEx Portal: TUSC7. https://www.gtexportal.org/home/gene/TUSC7. Accessed October 12, 2022.
43. Ren W, Chen S, Liu G, Wang X, Ye H and Xi Y: TUSC7 acts as a tumor suppressor in colorectal cancer. Am J Transl Res 9: 4026-4035, 2017.
44. Cong M, Li J, Jing R and Li Z: Long Non-Coding RNA tumor suppressor candidate 7 functions as a tumor suppressor and inhibits proliferation in osteosarcoma. Tumour Biol 37: 9441-9450, 2016.
45. Zheng BH, He ZX, Zhang J, Ma JJ, Zhang HW, Zhu W, Shao ZM and Ni XJ: The biological function of TUSC7/MiR-1224-3p axis in triple-negative breast cancer. Cancer Manag Res 13: 5763-5774, 2021.
46. Darb-Esfahani S, Denkert C, Stenzinger A, Salat C, Sinn B, Schem C, Endris V, Klare P, Schmitt W, Blohmer JU, *et al*: Role of TP53 mutations in triple negative and HER2-Positive breast cancer treated with neoadjuvant Anthracycline/Taxane-Based chemotherapy. Oncotarget 7: 67686-67698, 2016.
47. GTEx Portal: HNF1A-AS1. https://www.gtexportal.org/home/gene/HNF1A-AS1. Accessed February 1, 2023.
48. Liu Y, Zhao F, Tan F, Tang L, Du Z, Mou J, Zhou G and Yuan C: HNF1A-AS1: A Tumor-associated long non-coding RNA. Curr Pharm Des 28: 1720-1729, 2022.
49. Zhang G, An X, Zhao H, Zhang Q and Zhao H: Long non-coding RNA HNF1A-AS1 promotes cell proliferation and invasion via regulating MiR-17-5p in Non-Small cell lung cancer. Biomed Pharmacother 98: 594-599, 2018.
50. Liu L, Chen Y, Li Q and Duan P: LncRNA HNF1A-AS1 modulates non-small cell lung cancer progression by targeting MiR-149-5p/Cdk6. J Cell Biochem 120: 18736-18750, 2019.
51. Zhang Y, Shi J, Luo J, Liu C and Zhu L: Regulatory mechanisms and potential medical applications of HNF1A-AS1 in cancers. Am J Transl Res 14: 4154-4168, 2022.
52. Zhou X, Fan YH, Wang Y and Liu Y: Prognostic and clinical significance of long non-coding RNA HNF1A-AS1 in solid cancers: A systematic review and meta-analysis. Medicine (Baltimore) 98: e18264, 2019.
53. Shi Y, Zhang Q, Xie M, Feng Y, Ma S, Yi C, Wang Z, Li Y, Liu X, Liu H, *et al*: Aberrant Methylation-mediated decrease of LncRNA HNF1A-AS1 contributes to malignant progression of laryngeal squamous cell carcinoma via EMT. Oncol Rep 44: 2503-2516, 2020.
54. Ding CH, Yin C, Chen SJ, Wen LZ, Ding K, Lei SJ, Liu JP, Wang J, Chen KX, Jiang HL, *et al*: The HNF1α-Regulated LncRNA HNF1A-AS1 reverses the malignancy of hepatocellular carcinoma by enhancing the phosphatase activity of SHP-1. Mol Cancer 17: 63, 2018.
55. Dang Y, Lan F, Ouyang X, Wang K, Lin Y, Yu Y, Wang L, Wang Y and Huang Q: Expression and clinical significance of long Non-Coding RNA HNF1A-AS1 in human gastric cancer. World J Surg Oncol 13: 302, 2015.
56. Kanduri C: Long Noncoding RNAs: Lessons from genomic imprinting. Biochim Biophys Acta 1859: 102-111, 2016.
57. BioGPS: IPW (Imprinted in Prader-Willi Syndrome). http://biogps.org/#goto=genereport&id=3653. Accessed October 12, 2022.
58. Ma B, Li Y and Ren Y: Identification of a 6-lncRNA prognostic signature based on microarray Re-annotation in gastric cancer. Cancer Med 9: 335-349, 2019.
59. Tang SJ, You GR, Chang JT and Cheng AJ: Systematic analysis and identification of dysregulated panel LncRNAs contributing to poor prognosis in Head-neck cancer. Front Oncol 11: 731752, 2021.
60. GeneCards - The Human Gene Database: CLIP4. https://www.genecards.org/cgi-bin/carddisp.pl?gene=CLIP4&keywords=CLIP4 Accessed February 2, 2023.
61. Park JS, Pierorazio PM, Lee JH, Lee HJ, Lim YS, Jang WS, Kim J, Lee SH, Rha KH, Cho NH and Ham WS: Gene expression analysis of aggressive clinical T1 stage clear cell renal cell carcinoma for identifying potential diagnostic and prognostic biomarkers. Cancers (Basel) 12: E222, 2020.
62. Ahn J, Han KS, Heo JH, Bang D, Kang YH, Jin HA, Hong SJ, Lee JH and Ham WS: FOXC2 and CLIP4: A potential biomarker for synchronous metastasis of ≤7-Cm clear cell renal cell carcinomas. Oncotarget 7: 51423-51434, 2016.
63. Gong A, Zhao X, Pan Y, Qi Y, Li S, Huang Y, Guo Y, Qi X, Zheng W and Jia L: The LncRNA MEG3 mediates renal cell cancer progression by regulating ST3Gal1 transcription and EGFR sialylation. J Cell Sci 133: jcs244020, 2020.
64. He H, Dai J, Zhuo R, Zhao J, Wang H, Sun F, Zhu Y and Xu D: Study on the mechanism behind LncRNA MEG3 affecting clear cell renal cell carcinoma by regulating MiR-7/RASL11B signaling. J Cell Physiol 233: 9503-9515, 2018.
65. Cheng T, Shuang W, Ye D, Zhang W, Yang Z, Fang W, Xu H, Gu M, Xu W and Guan C: SNHG16 promotes cell proliferation and inhibits cell apoptosis via regulation of the MiR-1303-p/STARD9 Axis in clear cell renal cell carcinoma. Cell Signal 84: 110013, 2021.