CLINICAL STUDY

NDUFA4L2 expression predicts poor prognosis in clear cell renal cell carcinoma patients

Lei Liu, Gongbin Lan, Longkai Peng, Xubiao Xie, Fenghua Peng, Shaojie Yu, Yu Wang and Xiaotian Tang

Department of Urological Organ Transplantation, The Second Xiangya Hospital, Central South University, Changsha, China

ABSTRACT

Background: NDUFA4L2 is overexpressed in VHL-deficient cell lines and neuroblastoma. The clinical significance of NDUFA4L2 in clear cell renal cell carcinoma (ccRCC) has not been well studied. Therefore, we evaluated the prognostic value of NDUFA4L2 in ccRCC patients.

Methods: In our study, NDUFA4L2 expression in 86 cases of ccRCC and adjacent normal tissues was monitored by immunohistochemistry, semi-quantitative RT-PCR, and Western blot analyses. The relationship between NDUFA4L2 expression and the clinical features of ccRCC was assessed.

Results: The results showed that NDUFA4L2 protein expression was found to be higher in ccRCC tissues 81.4% (70/86) than in normal tissues 26.7% (23/86) \( (p = 0.021) \). The average level of NDUFA4L2 mRNA expression was found to be 122.23 \( \pm \) 6.018 and 21.34 \( \pm \) 1.036 in ccRCC tissue and adjacent normal tissue \( (p < 0.001) \). NDUFA4L2 expression levels were correlated with some clinical features of ccRCC. Multivariate analysis showed NDUFA4L2 expression was an independent prognostic factor for ccRCC patients.

Conclusions: Our study has provided the significant clinical relevance of NDUFA4L2 in ccRCC and suggested that ccRCC patients with NDUFA4L2 overexpression may be suitable as a potential therapeutic target for ccRCC patients.

ARTICLE HISTORY

Received 28 February 2016
Revised 22 May 2016
Accepted 24 June 2016
Published online 20 July 2016

KEYWORDS

Clear cell renal cell carcinoma; NDUFA4L2; biomarker; prognosis

Introduction

Renal cell carcinoma (RCC) is the most common primary tumor arising from the kidney in adults, accounting for more than 85% of all renal malignancies, with clear cell renal cell carcinoma (ccRCC) representing approximately 75% of all RCCs.\(^1\)-\(^3\) Approximately 10–28% of RCC will develop a local recurrence or distant metastasis after curative nephrectomy. Metastatic RCC is resistant to many therapies including radiotherapy and chemotherapy. Although recently developed targeted therapies for advanced RCC have proved certain improvement in selective patients, the majority of advanced RCC patients remain refractory to the treatment.\(^4\) Approximately a quarter of patients with RCC will develop a metastatic disease despite curative surgical removal of the primary tumor.\(^5\) The overall 5-year survival rate of RCC clinic patients ranges from 5% to 10%.\(^6\) Thus, understanding molecular mechanisms of RCC progression in order to identify new targets for a new and better therapy is still needed.\(^6,7\)

The mitochondrial NDUFA4L2 (NADH dehydrogenase [ubiquinone] 1 alpha sub complex, 4-like 2, also called NADH-ubiquinone oxidoreductase MLRQ subunit homolog, NUOMS), a negative regulator of mitochondrial complex 1, has identified as a HIF-1 target gene and localized in mitochondria.\(^8\) It was previously described using mRNA array technology that NDUFA4L2 is overexpressed in VHL-deficient cell lines and tumors,\(^9,10\) as well as in neuroblastoma cells in hypoxia\(^11\) and in pathophysiological conditions like rheumatoid arthritis.\(^12\) Currently, the correlation between NDUFA4L2 gene expression and ccRCC still remains elusive. Recently, Minton et al.\(^13\) have reported that NDUFA4L2 was overexpression in ccRCCs and the over-regulation of NDUFA4L2 was associated with an unfavorable patients’ prognosis. Considering the relatively small number of analyzed patients in the study of Minton et al. Further validation studies are needed before application of NDUFA4L2 in the clinical setting.

In this study, we identified NDUFA4L2 as one of the most significantly overexpression genes in ccRCC tissues. We also evaluated the correlations of NDUFA4L2 expression with the clinicopathological variables and clinical outcome in patients with ccRCC. In addition, our data based on clinical ccRCC tissues indicated that
NDUFA4L2 might be a potential biomarker for discriminating between ccRCC and normal tissues.

**Materials and methods**

**Clinical data**

From December 2009 to December 2012, surgical specimens (paired normal and cancerous tissues) were obtained from 86 consecutive patients in Second Xiangya Hospital, The Central South University. There were 34 females and 52 males, aged from 29 to 75 years old, with a median age of 54 years. All patients were pathologically diagnosed to have ccRCC and staged in accordance with the latest tumor-node-metastasis (TNM) classification system. All patients did not have any neoadjuvant therapies before surgery. The fresh specimens of tumor tissue or adjacent normal tissue were immediately taken after the surgery, one was freshly frozen in liquid nitrogen and stored at $-80^\circ\text{C}$ for RNA and protein extraction, and the other one was fixed in 4% paraformaldehyde solution, and then embedded in paraffin for immunohistochemistry. Follow-up data were obtained by outpatient clinical database, phone interview, and postal letter communication. The clinicopathological characteristics of these patients were collected from the medical records and are summarized in Table 1. All patients were monitored from the date of initial surgery until death or the closing date of this study (30 September 2014). The mean follow-up time period was 42.5 months (range between 6 and 65 months).

**Immunohistochemistry**

To detect the expression of NDUFA4L2 proteins, formalin-fixed and paraffin-embedded tissue sections were immunostained with a polyclonal anti-NDUFA4L2 antibody (at 1:100 dilution; OriGene, Rockville, MD) using standard techniques. The immunostained sections were then evaluated in accordance with a previous study. Briefly, all tissue sections were reviewed under a light microscopy and scored for at least five fields at a 400× magnification independently by two pathologists who were unaware of any clinical or outcome data.

**Quantitative real-time RT-PCR**

Total RNA was extracted from the ccRCC and normal tissue using TRIzol reagent (Invitrogen; Life Technologies, Gaithersburg, MD) the manufacturer’s protocol. The RT-PCR assays were performed using the TaqMan Reverse Transcription Kit (Applied Biosystems, Foster City, CA) and the 7500 Fast Real-Time PCR System (Applied Biosystems) for quantitative RNA detection, and each RNA TaqMan PCR probe was purchased from Applied Biosystems. The real-time RT-PCR assays were performed using the 7500 Fast Real-Time PCR System for quantitative mRNA detection and with iTaq Fast SYBR Green Supermix (Bio-Rad, Hercules, CA).

**Western blot**

All operations were completed on the ice. At 4°C, 12,000 r/min centrifugation lasted for 20 min, and then the supernatant was taken for backup at $-20^\circ\text{C}$. After the detection of protein concentration with BCA Protein Assay Kit, each hole was given a sample amount of 50 μg for SDS-PAGE. After blocking membranes, they were incubated with appropriate dilutions of specific primary antibodies, the blots were incubated with HRP-conjugated secondary antibodies and visualized using ECL system (Thermo Fisher Scientific, Rochester, NY). Western blot data were quantified by normalizing the signal intensity of each sample to that of β-actin.

**Statistical analyses**

Statistical analyses were performed using SPSS software version 16.0 for Microsoft Windows (SPSS Inc., Chicago, IL). The chi-square ($\chi^2$) test was used to assess the correlations between NDUFA4L2 expression and clinicopathological characteristics. Overall/tumor-free survival of patients was stratified using the Kaplan–Meier method and statistically analyzed using the log rank test. Univariate and multivariate analyses using Cox proportional hazard models were conducted to measure...
correlations between clinicopathological factors and survival probabilities. Values of $p < 0.05$ were considered to be statistically significant.

**Ethics statement**

This study was reviewed and approved by the Ethics Committee of The Central South University (No. 110021970), and an informed consent form was signed by each patient before surgery.

**Results**

**Expression of NDUFA4L2 is increased in RCC tissues**

We first analyzed the expression of NDUFA4L2 in 86 human ccRCC tissues with paired adjacent normal tissues by immunohistochemistry, qRT-PCR, and Western blotting analysis, respectively. In normal kidney tissues, NDUFA4L2 staining was negative or weak (Figure 1(A)). In ccRCC tissues, NDUFA4L2 staining ranged from light yellow to brown (Figure 1(B)). According to the above-described criteria, NDUFA4L2 overexpression was observed in 81.4% (70/86) of renal tumor samples, but only in 26.7% (23/86) of adjacent normal tissues. Interestingly, real-time PCR indicated that NDUFA4L2 mRNA in ccRCC tissues was found to be dramatically higher than in normal tissues, $122.23 \pm 6.018$ and $21.34 \pm 1.036$, respectively. The difference was found to be statistically significant ($p < 0.001$) (Figure 1(C)). The results above imply that NDUFA4L2 was overexpression in ccRCC, in keeping with the previous study, Western blotting results clearly indicated that the ccRCC cancer tissue had a drastic increase of NDUFA4L2 expression as compared with the corresponding normal tissues.

![Figure 1. Expressions of NDUFA4L2 in human ccRCC tissues and matched non-cancer tissues. (A) Negative or low-level staining of NDUFA4L2 in paired normal tissues; (B) Strong staining in majority of ccRCC tissue, magnification: 400×; Expressions of NDUFA4L2 mRNA (C) and protein (D, E) in representative pairs of matched normal renal tissues (N) and ccRCC tissues (C) were assessed by Western blotting.](image-url)
(Figure 1(D,E)), which indicated NDUFA4L2 expression was associated with the aggressive phenotypes of ccRCC.

**Association of NDUFA4L2 expression with clinicopathological features from ccRCC patients**

We further analyzed the relationship between clinicopathological features and NDUFA4L2 expression levels in ccRCC cases (Table 1). A total of 70 (81.4%) cases showed high levels of NDUFA4L2 expression (score 3–6). Our result revealed that increasing increased NDUFA4L2 expression correlated with depth of invasion \( (p = 0.01) /\) Fuhrman grade \( (p = 0.042) /\) lymph node status \( (p = 0.034) \) and distant metastasis \( (p = 0.019) \). However, no significant association was found between NDUFA4L2 expression and other clinicopathological features, including age, gender, primary tumor size, and TNM stage (Table 1). These findings strongly indicated that NDUFA4L2 expression plays a critical role in ccRCC development and progression and is a valuable biomarker for this disease.

**Increased expression of NDUFA4L2 correlated with the poor prognosis ccRCC patients**

To assess the clinical significance of NDUFA4L2 overexpression in ccRCC, we analyzed the relationship between the level of NDUFA4L2 expression and patient survival. Follow-up data ended in 30 September 2014 after a revisit time of 65 months. The mean follow-up time period was 42.5 months (range between 6 and 65 months). An estimated 5-year survival rate was 48.9%. Among all cases, 60 were still alive and 26 were dead. All the enrolled patients were divided into two groups according to NDUFA4L2 expression level. There were 70 patients with high levels of NDUFA4L2 expression, among whom 45 were still alive and 25 were dead. The survival rate was 64.3%. 25 (32.9%) patients in high NDUFA4L2 protein expression group died, while only 1 (6.2%) died among those with low NDUFA4L2 protein expression. The overall survival time of patients with high NDUFA4L2 expression was shorter than those with low expression according to Kaplan–Meier analysis \( (p = 0.016) \) (Figure 2). During the 65 months of follow-up, 59 cases were non-recurrent and 27 cases were recurrent. We further examined the tumor-free survival of NDUFA4L2-negative and positive groups and observed a statistically significant difference between the two groups using the log-rank test. The survival of NDUFA4L2-negative patients was longer than that observed for NDUFA4L2-positive patients \( (p = 0.046) \) (Figure 3). The uni- and multivariate analyses indicated that expression of NDUFA4L2 was independent predictors for tumor-free survival of ccRCC patients (Table 2). The univariate analysis indicated that among the depth of invasion, Fuhrman grade, lymph node/distant metastasis correlated with the outcome of ccRCC patients. Further assessment using the Cox's multivariate analysis showed that depth of invasion, Fuhrman grade, distant metastasis, and NDUFA4L2 expression were statistically significant predictors for survival of ccRCC patients (Table 2).

**Discussion**

Seven genes commonly mutated in human ccRCC, including VHL (NCBI Gene ID: 7428), MET (4233), FLCN (201163), TSC1 (7248), TSC2 (7249), FH (2271), and SDH (6390, 6391, and 6392), have been identified to date.16 Interestingly, all seven genes are involved in the regulation of metabolic pathways. These data support the
theory that kidney cancer is a metabolic disease. Loss of expression or mutation of the von Hippel–Lindau (VHL) tumor suppressor gene is found in hereditary and most sporadic ccRCCs. This suggests an etiological role for VHL gene loss in renal carcinogenesis. However, the exact pathway by which loss of VHL leads to ccRCC has not been definitively elucidated. The best studied and likely most important effect of VHL loss is the result of expression or mutation of the von Hippel–Lindau (VHL) tumor suppressor gene is found in hereditary and most sporadic ccRCCs. This suggests an etiological role for VHL gene loss in renal carcinogenesis. However, the exact pathway by which loss of VHL leads to ccRCC has not been definitively elucidated. The best studied and likely most important effect of VHL loss is the result of expression or mutation of the von Hippel–Lindau (VHL) tumor suppressor gene is found in hereditary and most sporadic ccRCCs. This suggests an etiological role for VHL gene loss in renal carcinogenesis. However, the exact pathway by which loss of VHL leads to ccRCC has not been definitively elucidated. The best studied and likely most important effect of VHL loss is the result

| Characteristics          | Univariate analysis | Multivariate analysis |
|--------------------------|---------------------|-----------------------|
|                          | HR (95% CI)         | p Value               | HR (95% CI)         | p Value               |
| Age (years)              |                     |                       |                     |                       |
| <50                      | 1                   |                       | 1                   |                       |
| ≥50                      | 1.14 (0.85–1.79)    | 0.326                 | 1.27 (0.73–1.88)    | 0.432                 |
| Gender                   |                     |                       |                     |                       |
| Male                     | 1                   |                       | 1                   |                       |
| Female                   | 1.27 (0.73–1.88)    | 0.432                 | 1.62 (0.98–2.23)    | 0.028                 |
| Tumor size (cm)          |                     |                       |                     |                       |
| ≤7.0                     | 1                   |                       | 1                   |                       |
| >7.0                     | 0.87 (0.63–1.74)    | 0.312                 | 1.62 (0.98–2.23)    | 0.028                 |
| Depth of invasion        |                     |                       |                     |                       |
| T1 + T2                  | 2.43 (0.91–3.65)    | 0.001                 | 2.35 (0.54–2.66)    | 0.012                 |
| T3 + T4                  | 2.43 (0.91–3.65)    | 0.001                 | 2.35 (0.54–2.66)    | 0.012                 |
| Fuhrman grade            |                     |                       |                     |                       |
| G1–2                     | 1                   |                       | 1                   |                       |
| G3–4                     | 1.82 (0.87–3.12)    | 0.031                 | 2.45 (1.25–3.95)    | 0.042                 |
| TNM stage                |                     |                       |                     |                       |
| I + II                   | 1                   |                       | 1                   |                       |
| III + IV                 | 2.26 (1.13–2.78)    | 0.066                 | 1.62 (0.98–2.23)    | 0.028                 |
| Lymph node status        |                     |                       |                     |                       |
| Negative                 | 1                   |                       | 1                   |                       |
| Positive                 | 1.62 (0.98–2.23)    | 0.028                 | 1.38 (1.15–2.88)    | 0.063                 |
| Distant metastasis       |                     |                       |                     |                       |
| Absent                   | 1                   |                       | 1                   |                       |
| Present                  | 2.34 (0.89–3.89)    | 0.002                 | 1.75 (1.33–3.22)    | 0.022                 |
| NDUFA4L2 expression      |                     |                       |                     |                       |
| Low                      | 1                   |                       | 1.14 (0.85–1.79)    | 0.326                 |
| High                     | 2.89 (1.67–4.42)    | 0.001                 | 1.97 (0.89–3.98)    | 0.002                 |

overexpressed in human ccRCC specimens, which was significantly associated with poorly differentiated ccRCC. In addition, the survival analysis in the current study showed that the tumor-free/overall survival rate for patients in the NDUFA4L2-positive expression group was significantly lower than that of the NDUFA4L2-negative group.

To study the specific role of HIF-1a and HIF-2a in the response of NDUFA4L2 to hypoxia, the previous study performed RNA interference assays in human renal carcinoma RCC4 cells that constitutively stabilize HIF-1a and HIF-2a through the absence of VHL. In line with the role of HIF activity in NDUFA4L2 regulation, there was a robust upregulation of NDUFA4L2 in hypoxic RCC4/VHL cells, whereas there was marked and constitutive NDUFA4L2 expression in normoxic RCC4 cells that was only minimally upregulated in hypoxic conditions.

In our study, we first detected an expression NDUFA4L2 in ccRCC versus normal kidney tissues for biomarker discovery. Overexpression of the NDUFA4L2 protein was associated with advanced ccRCC depth of invasion/ Fuhrman grade/lymph node metastasis and distance metastasis but was not associated with age/gender/tumor size and TNM stage. The uni- and multivariate analyses demonstrated that expression of NDUFA4L2 is an independent predictor for tumor-free survival of ccRCC patients. These results suggest that NDUFA4L2 is crucially implicated in the carcinogenesis and invasion of ccRCC and may be further evaluated as a biomarker for the prediction of ccRCC progression and tumor-free survival.

Perhaps the best-studied example of chronic hypoxia is the hypoxia associated with the tumor microenvironment. The tumor suffers from poor oxygen supply through a chaotic jumble of blood vessels that are unable to adequately perfuse the tumor cells. Hypoxia elicits a series of pro-tumorigenic responses through HIF-1 as well. New evidence suggests that ccRCC is a metabolic disease. All known kidney cancer susceptible genes are involved in the regulation of metabolic pathways. ccRCC cells contain increased amounts of glycogen and lipid in their cytoplasm. Previous study shows that NDUFA4L2 is the gene overexpressed to the greatest extent in the human ccRCC datasets in Oncomine. However, its expression in human cancer has not yet been explored. In the present study, immunohistochemistry, Western blot analysis, and semi-quantitative RT-PCR all demonstrated that NDUFA4L2 was highly expressed in ccRCC patients, but little or no expression was detected in normal tissue.

NDUFA4L2 molecules are transmembrane glycoproteins expressed on the surface of most mitochondrial.
On the other side, cancer-associated fibroblasts (CAFs) provide critical metabolites for tumor growth and undergo metabolic reprogramming to support glycolysis.\textsuperscript{14} Increasing evidence suggests that CAFs can also secrete metabolites to fuel the growth of tumor cells.\textsuperscript{24} However, the molecular mechanisms responsible for this change remain unclear. Zhang et al. provide conclusive evidence that CAFs are prone to glycolysis and that this metabolic change is responsible for the tumor-promoting effect of CAFs. In addition, their study identifies that overexpression NDUFA4L2 can inhibit oxidative phosphorylation, which, in turn, promotes glycolysis in CAFs.\textsuperscript{14} Nonetheless, further studies are still required to precisely understand the molecular mechanism of NDUFA4L2 inactivation in the development and progression of ccRCC and other human tumors. Moreover, in the present study, we found that immunoreactivity of NDUFA4L2 was observed primarily in the cytoplasm of tumor tissues in some cases.

Currently, gene therapy shows great value in the treatment of ccRCC, but only a few new genes have been applied to clinical treatment, and key therapeutic targets have not yet been found.\textsuperscript{9,23} Latest results show that NDUFA4L2 was drastically overexpressed in human hepatocellular carcinoma and induced by hypoxia. Inactivation of HIF1/NDUFA4L2 increased mitochondrial activity and oxygen consumption, resulting in ROS accumulation and apoptosis.\textsuperscript{21} Our study shows high levels of NDUFA4L2 gene expression in ccRCC patients. Our results also indicate NDUFA4L2 was an independent predictor for survival of ccRCC patients. It provides a new target for ccRCC therapy. However, knowledge about NDUFA4L2, particularly on its roles in cancer development, is scarce. The mechanism or pathway by which NDUFA4L2 affects the incidence and development of ccRCC merits further investigation.

The limitations of our study include: first, the patients recruited into this study were not given the same chemotherapy regimen both in terms of schedule and associated drug. Second, the relatively small number of analyzed patients, which may reduce the power to detect statistical associations and significantly affect survival analyses. Finally, beyond cause of mortality, data on cancer recurrences were not available in these cohorts. Therefore, further investigations are still required to confirm our results in a larger series with a uniform treatment protocol.

**Disclosure statement**

The authors have declared that no competing interests exist.

**References**

1. Znaor A, Lortet-Tieulent J, Laversanne M, et al. International variations and trends in renal cell carcinoma incidence and mortality. *Eur Urol*. 2014;67:519–630.

2. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA Cancer J Clin*. 2014;64:9–29.

3. Jonasch E, Gao J, Rathmell WK. Renal cell carcinoma. *BMJ*. 2014;349:g4797.

4. Motzer RJ, Hutson TE, Tomczak P, et al. Sunitinib versus interferon alpha in metastatic renal-cell carcinoma. *N Engl J Med*. 2007;356:115–124.

5. Grimm MO, Wolff I, Zastrow S, et al. Advances in renal cell carcinoma treatment. *Ther Adv Urol*. 2010;2:11–17.

6. Cohen HT, McGovern FJ. Renal-cell carcinoma. *N Engl J Med*. 2005;353:2477–2490.

7. Rini BI, Campbell SC, Escudier B. Renal cell carcinoma. *Lancet*. 2009;373:1119–1132.

8. Tello D, Balsa E, Acosta-Iborra B, et al. Induction of the mitochondrial NDUFA4L2 protein by HIF-1ction of es oxygen consumption by inhibiting complex I activity. *Cell Metab*. 2011;14:768–779.

9. Papandreou I, Cairns RA, Fontana L, et al. HIF-1 mediates adaptation to hypoxia by actively downregulating mitochondrial oxygen consumption. *Cell Metab*. 2006;3:187–197.

10. Favier J, Briere JJ, Burnichon N, et al. The Warburg effect is genetically determined in inherited pheochromocytomas. *PLoS One*. 2009;4:e7094.

11. Fredlund E, Ovenberger M, Borg K, Pahlman S. Transcriptional adaptation of neuroblastoma cells to hypoxia. *Biochem Biophys Res Commun*. 2006;337:1054–1060.

12. Andreas K, Haupl T, Lubke C, et al. Antiinflammatory drug response signatures in human chondrocytes: Potential molecular targets to stimulate cartilage regeneration. *Arthritis Res Ther*. 2009;11:R15.

13. Minton DR, Fu L, Morgan NP, et al. Role of NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4-like 2 in clear cell renal cell carcinoma. *Clin Cancer Res*. 2016;22:2791–2801.

14. Zhang D, Wang Y, Shi Z, et al. Metabolic reprogramming of cancer-associated fibroblasts by IDH3alpha downregulation. *Cell Rep*. 2015;10:1335–1348.

15. Liu S, Qi L, Han W, et al. Overexpression of wip1 is associated with biologic behavior in human clear cell renal cell carcinoma. *PLoS One*. 2014;9:e110218.

16. Linehan WM, Srinivasan R, Schmidt LS. The genetic basis of kidney cancer: A metabolic disease. *Nat Rev Urol*. 2010;7:277–285.

17. Linehan WM, Pinto PA, Srinivasan R, et al. Identification of the genes for kidney cancer: Opportunity for disease-specific targeted therapeutics. *Clin Cancer Res*. 2007;13:671s–679s.

18. Maher ER, Kaelin WG Jr. von Hippel-Lindau disease. *Medicine (Baltimore)*. 1997;76:381–391.

19. Semenza GL. Defining the role of hypoxia-inducible factor 1 in cancer biology and therapeutics. *Oncogene*. 2010;29:625–634.

20. Moeller BJ, Cao Y, Li CY, Dewhirst MW. Radiation activates HIF-1 to regulate vascular radiosensitivity.
in tumors: Role of reoxygenation, free radicals, and stress granules. *Cancer Cell*. 2004;5:429–441.

21. Lai RK, Xu IM, Chiu DK, et al. NDUFA4L2 fine-tunes oxidative stress in hepatocellular carcinoma. *Clin Cancer Res*. 2016;22:3105–3117.

22. Krishnan B, Truong LD. Renal epithelial neoplasms: The diagnostic implications of electron microscopic study in 55 cases. *Hum Pathol*. 2002;33:68–79.

23. Fu L, Minton DR, Zhang T, et al. Genome-wide profiling of TRACK kidneys shows similarity to the human ccRCC transcriptome. *Mol Cancer Res*. 2015;13:870–878.

24. Fiaschi T, Marini A, Giannoni E, et al. Reciprocal metabolic reprogramming through lactate shuttle coordinately influences tumor-stroma interplay. *Cancer Res*. 2012;72:5130–5140.