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Research Article

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Essential Elements as Biomarkers of Metastatic Renal Cell Carcinoma

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Abstract

Renal cell carcinoma (RCC) represents 3% of human malignant tumors and approximately 90% of malignant renal neoplasms. Despite great therapeutic advances in the last decade, metastatic RCC (mRCC) is still considered an incurable disease. In this study, we examined the potential of essential elements as biomarkers of mRCC using an orthotropic metastatic mouse model. Frozen lung and plasma samples from healthy and mRCC-induced mice were lyophilized, digested, and analyzed using inductively coupled plasma mass spectrometry. In metastatic lungs, a significant increase in Ca concentration (268%) was observed, whereas a significant decrease in Cu (23.2%), Fe (17.4%), Mn (38.8%), and Na (11.7%) was observed. The plasma of mRCC-induced mice showed decreased concentrations of Mn (53%), Na (19.7%) and Zn (49,50%) and increased levels of Ca (53%), Cu (39.5%), Our findings revealed marked differences in the concentrations of essential elements in the lung and plasma of the metastatic mouse model. The circulating levels of Ca, Cu, Mn, Na, and Zn could be utilized as diagnostic and therapeutic response biomarkers.

Keywords: Essential Elements, Renal Cell Carcinoma, ICP-MS, Cell Metabolism, Malignant Transformation

Introduction

Renal cell carcinoma (RCC) is one of the most lethal urological malignancies because of its aggressive behavior [1]. Among a variety of histological subtypes, clear cell RCC is the most common subtype of RCC, accounting for 75% of all cases. Mutations in the Von Hippel-Lindau (VHL) tumor suppressor are frequently found in clear cell RCC, and a deficiency in the VHL protein stabilizes hypoxia-inducible factor 1α (HIF-1α), which in turn leads to increased angiogenesis [2].

Surgical resection is the mainstay option for localized RCC. However, 20–40% of patients will experience relapse and develop metastatic disease, and the lung is the most common metastatic site (approximately 50% of cases) [3, 4]. The therapeutic management of metastatic RCC (mRCC) has changed dramatically in the last decade. Advances in molecular and cellular biology have led to the development of drugs capable of blocking specific cell signaling pathways. The use of these agents, such as antagonists of vascular endothelial growth factor signaling and blockers of the mammalian target of rapamycin pathway, is known target therapy [5]. The use of molecular-targeted therapy has intensified the need to identify diagnostic and prognostic biomarkers [6]. Tissue, urine, and blood have been used to identify diagnostic and prognostic biomarkers for mRCC, but scientific data supporting their routine clinical application remain insufficient [7].

Essential elements are important factors mediating the biochemical processes that guarantee homeostasis in the body. An imbalance in the concentration of essential elements could indicate the presence of a disease [8]. Animal models are valuable tools for studying tumor biology and investigating anti-cancer therapeutics and biomarkers [9, 10]. The aim of this study was to examine the concentration of essential elements in animal models of mRCC.

Material and Methods

Plasma and Tissue Sample Preparation

Frozen plasma and lung samples were obtained from archived tumor samples in our laboratory (Animal Experimentation Ethics Committee; number of processes: 87/11). These samples were obtained in a previous study where BalbC mice were orthotopically injected with Renca cells (murine RCC cells) that then developed metastasis [5].

Lyophilization

Lyophilization was performed using Dura Dry (FD2085B0000) and Dura Stop (TDS3BOT5000) (FTS Systems) freeze-dryers. The process was controlled by the lyophilizer using a program that takes into account the eutectic point of the sample (i.e. the lowest temperature that can be obtained during the freezing of a solution), as follows:

F (280 min): Freezing time of the sample on the shelf at -40°C.
P (260 min): Primary freeze-drying time, with shelf at −22°C and vacuum at 200 mTorr.
S (230 min): Secondary freeze-drying time (removal of moisture residues), with shelf at +20°C and vacuum at 10 mTorr.
T (770 min): total processing time.

Sample Preparation and Determination of Trace Elements

Sample preparation was previously described in detail by Bellini et al [11]. In brief, samples were digested in a microwave oven, and trace elements were identified using an inductively coupled plasma mass spectrometer (ICP-MS) equipped with a reaction cell (ICP-MS 7700, Hachioji, Japan) and operated with high-purity argon (99.99%, White, Brazil) and helium (99.99%, White, Brazil).

Histological Analysis

Paraffin-embedded samples from our archive were used for histological analysis, which was performed on 4-µm sections stained with hematoxylin and eosin (HE).

Statistical analysis

Simple comparisons were made using the F-test and Student's t-test, and multiple comparisons were performed using one-way analysis of variance followed by Bonferroni's test. A P value of less than 0.05 was considered statistically significant. The experiments were performed in triplicate and the results are presented as the mean ± standard error (SE) of single comparisons.

Results

In this work, lung tissue and plasma samples of normal (control) and mice bearing metastatic tumors were analyzed. MRCC is life threatening because of the rapid proliferation of tumor cells and severe destruction of the lung. Figure 1A shows normal lung parenchyma, while Figure 1B shows a number of metastatic foci in the lung parenchyma.

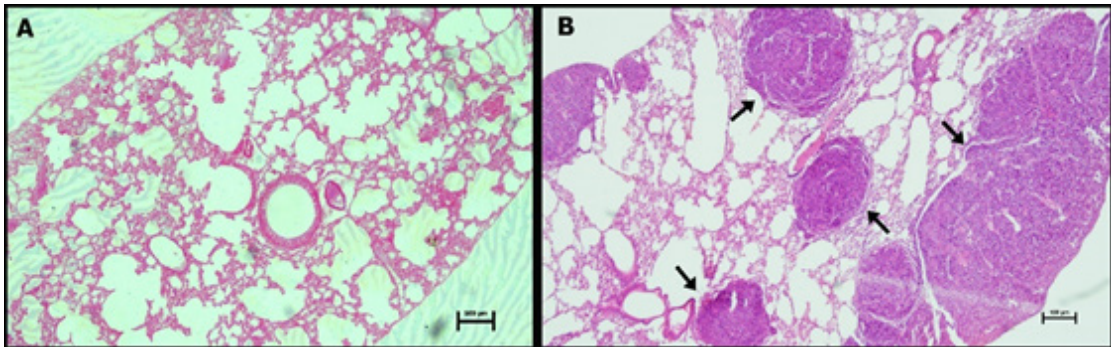

Figure 1: Microscopic view of HE-stained lung tissues. (A) Control: normal lung parenchyma. (B) Metastatic lung: tumor nodules observed within the lung tissue (arrows). Magnification: 40×, Nikon Eclipse Ci (Nikon; Tokyo; Japan).

The presence of metastatic cells in the lung parenchyma impacts the biochemical profile of the lung in other systems of the body, promoting significant changes in cellular composition and functionality. Such changes lead to the emergence of biomarkers.

Initially, we analyzed the concentrations of essential elements in normal and metastatic lungs. Of the 11 elements analyzed, five showed significant differences in concentration between the groups. The most striking difference was the 268% increase in Ca concentration in metastatic lungs. On the contrary, a significant decrease was observed for Cu (23.2%), Fe (17.4%), Mn (38.8%), and Na (11.7%) (Table 1).

Table 1. Comparison of the concentrations of trace elements (µg/g) between normal and lung metastasis-bearing mice.

Elements	Control	mRCC	P
Ca	54.66 ± 6.96	146.42 ± 18.62	<0.0001
Co	0.0607 ± 0.0044	0.0624 ± 0.0095	NS
Cr	0.2299 ± 0.007	0.2497 ± 0.1798	NS
Cu	10.42 ± 0.32	8.46 ± 1.02	<0.01
Fe	453.89 ± 40.91	386.64 ± 69.13	<0.05
K	12115.30 ± 256.30	13039.51 ± 24.48	NS
Mg	573.51 ± 19.61	629.41 ± 89.93	NS
Mn	0.8744 ± 0.0621	0.6301 ± 0.1152	<0.01
Na	6653.30 ± 381.75	5955.86 ± 538.76	<0.05
Se	1.26 ± 0.07	1.53 ± 0.26	NS
Zn	75.73 ± 3.36	69.07 ± 3.24	NS

NS: Statistical difference is not significant.

To identify a possible circulating biomarker for mRCC, mouse plasma samples were analyzed by ICP-MS and the results are shown in Table 2. Of the 11 elements analyzed, five showed significant differences in plasma concentration between the control and metastasis groups. In particular, the plasma of mRCC-induced mice showed decreased concentrations of Mn (53%) and Zn (49,50%) and increased levels of Ca (53%), Cu (39.5%), and Na (19.7%).

Table 2. Comparison of trace element concentrations (µg/g) in plasma samples of normal and metastatic mice.

Elements	Control	mRCC	P
Ca	176.69 ± 17.13	309.71 ± 28.89	<0.01
Co	0.14 ± 0.005	0.17 ± 0.04	NS
Cr	0.41 ± 0.10	0.39 ± 0.04	NS
Cu	9.35 ± 0.41	13.04 ± 1.85	<0.05
Fe	154.73 ± 20.10	174.15 ± 14.72	NS
K	521.52 ± 37.80	526.86 ± 118.22	NS
Mg	381.4 ± 60.90	521.12 ± 102.58	NS
Mn	0.41 ± 0.05	0.22 ± 0.09	<0.05
Na	52627.9 ± 1928.93	42281.01 ± 17052.9	<0.05
Se	3.94 ± 0.18	4.16 ± 0.96	NS
Zn	42.76 ± 8.32	31.85 ± 1.22	<0.05

NS: Statistical difference is not significant.

Discussion

Experimental disease models have become crucial tools for the development of new diagnostic and therapeutic strategies. In this study, tissue and circulating biomarkers were identified in lung and plasma samples from a mouse model of mRCC. The metastatic lung nodule microenvironment contains, in addition to tumor cells, other components such as infiltrating inflammatory cells, endothelial cells, and fibroblasts [12]. This microenvironment interacts with parenchyma tissue to support the survival and expansion of tumor cells and is accompanied by increased glycolysis, hypoxia, oxidative stress, and impairment of metabolic enzymes.

Our findings showed that the levels of Ca increased by 268% in metastatic lungs. This significant increase can be explained in part by the process of calcification in the pulmonary parenchyma, a phenomenon known as dystrophic calcification that requires an inflamed lung parenchyma [13]. In mRCC-bearing mice, the concentration of Cu in the lung was reduced. As a cofactor of approximately 30 enzymes, Cu is an important element in cell biology. In the tumor microenvironment, insufficient Cu bioavailability is caused by enhanced glycolysis, which is an adaptive response to hypoxia [14].

In normoxia, when Fe is available in the cell, prolyl hydroxylase domain enzymes enable the VHL complex to bind and ubiquitinate HIF, allowing HIF to degrade in the proteasome. In contrast, in hypoxia, where low levels of Fe are present, prolyl hydroxylase domain enzymes lose their activity. Thus, the transcription factor HIFα is not degraded and is translocated to the nucleus, leading to the expression of genes responsible for the survival of tumor cells [15]. Anti-oxidant enzymes defend cells from oxidative stress and their impaired activity contributes to tumor development and progression. Low levels of Mn can impact this anti-oxidant mechanism by reducing the concentration of magnesium superoxide dismutase, which is often dysregulated in cancers including RCC [16].

The increase in Na concentration in metastatic lungs in this study is in agreement with previous findings in the literature. Na ions are critical for the maintenance of cellular homeostasis. Na+/H+ exchange kinetics is altered in malignant cells, resulting in increased intracellular Na concentration [17]. Moreover, the tumor microenvironment undergoes metabolic alteration, pH deregulation, and increased vascularization, which affect the distribution of Na+ within cancer cells and the extracellular space [18]. Five of the 11 elements analyzed in the mouse plasma showed significant changes in concentration. Elevated levels of circulating Ca have been associated with high levels of inflammatory cytokines such as interleukin 6 and interleukin 1, which are associated with poor prognosis [19].

Elevated serum Cu levels were observed in patients with poor prognosis [20]. Cu is capable of supplying oncogenic enzymes, which play a role in tumor growth and metastasis, and the use of Cu chelators in animal models and patients has been shown to inhibit tumor progression [14]. Zn is an essential mineral for the structure and function of metalloenzymes and transcription factors and is involved in DNA replication and repair, response to oxidative stress, cell cycle regulation, and apoptosis. Reduced levels of circulating Zn have been linked to animal models of cancer [21]. Furthermore, patients with ovarian cancer, cervical cancer, and RCC showed lower serum Zn concentrations compared to those in healthy individuals [22-24].

Mn is required for the activity of several enzymes and is involved in regulating mechanisms against free radicals [25]. Decreased levels of Mn were observed in the serum of patients with RCC in comparison to those in healthy subjects [24]. Hyponatremia (low serum levels of Na) appears to be caused by chronic inflammation and has been reported to result in poor survival in many solid tumors. In addition to being a prognostic factor, hyponatremia is also a predictive factor for the lack of response in mRCC patients receiving therapy [26]. The findings of the present study revealed marked differences in the concentration of essential elements in the lung and plasma of the metastatic mouse model. In the metastatic lungs, alterations in the concentrations of Ca, Cu, Fe, Mn, and Na corroborate the dysregulation of molecular pathways present in RCC. This experimental model is a good tool for studying RCC, and circulating levels of Ca, Cu, Mn, Na, and Zn could be considered as diagnostic and therapeutic response biomarkers.

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