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Research Article

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Variations in Essential Elements After Malignant Transformation of Kidney Epithelial Tubular Cells

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Abstract

Cell line-based research is a valuable tool for the study of cancer physiopathology and the discovery of new drugs for use in clinical practice. In this study, inductively coupled plasma mass spectrometry (ICP-MS) was used to estimate Ca, Co, Cu, Fe, K, Mg, Mn, Na, P, S, Se, and Zn in epithelial tubular cells (HK-2) and kidney tumor cells (Caki-1 cells). The most relevant difference was a decrease in the contents of Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn. A significant accumulation of Co was also detected in Caki-1 cells. The fold change variation of each element concentration between HK-2 and Caki-1 cells was Ca (−0.40), Co (1.37), Cu (−0.68), Fe (−0.56), K (−0.40), Mg (−0.41), Mn (-0.54), Na (−0.33), P (−0.31), S (−0.26), and Zn (−0.73). These findings indicate that the elements mainly affect the metabolic pathways of epithelial kidney cells. Thus, our findings open a new avenue for RCC target therapy.

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

Introduction

Cell lines are in vitro model systems that have been used in many fields of cancer research. Their applications include the functional evaluation of genes, the understanding of cellular signaling networks, drug discovery, and molecular therapy [1]. In oncology, cell-based assays are used to understand the mechanisms and causes of cancer at the biological and molecular levels, as well as for predicting the performance of cancer drugs in phase II clinical trials [2].

Renal cell carcinoma (RCC) currently comprises about 2% of all human malignancies. Despite improvements in RCC diagnosis and recent advances in targeted treatment, RCC remains one of the most lethal urological malignancies [3]. The clear cell renal cell carcinoma (ccRCC) subtype accounts for approximately 75% of cases and originates from the malignant transformation of the proximal convoluted tubule epithelium [4]. In ccRCC cells, the hypoxia-inducible factor (HIF) protein results in increased tumor angiogenesis, which is involved in development and progression [5].

Malignant transformation can be defined as the accumulation of genetic alterations in normal cells, leading to uncontrolled and invasive cell proliferation and morphological and metabolic alterations [6]. Metabolic changes are the result of enzymatic malfunctions [7]. Metalloenzymes are proteins that have a metal as a cofactor; this metal ion is incorporated during enzyme synthesis and contributes to their function [8].

The elemental composition, in addition to characterizing, can also reveal the cellular metabolic status of cells. According to the abundance of each element in the cells, elements can be classified as macro-, trace, and microelements. They can also be classified as essential and non-essential elements. The main functions of essential trace elements are in cell structure, hormonal function regulation, and as enzyme cofactors. An imbalance in the composition of trace metals, which is essential for normal homeostasis, may cause diseases [9]. The aim of this study was to examine the concentrations of elements in normal human proximal convoluted tubule epithelium and malignant ccRCC.

Material and Methods

Cell lines

The Caki-1 cell line (ATCC® HTB-46 ™) was maintained in Dulbecco's modified Eagle's medium (DMEM) (Cultilab) supplemented with

10% fetal bovine serum (FBS) (Life Technologies, MD, USA), 300 µg/ml streptomycin, and 100 U/ml penicillin (Life Technologies). The HK-2 cell line (ATCC® CRL-2190 ™), comprising human cells from the renal proximal tubule, was cultured in DMEM + F12 medium (Life Technologies) in a 1:3 ratio supplemented with 10% FBS (Life Technologies), 300 µg/ml streptomycin, and 100 U/ml penicillin (Life Technologies). Cells were maintained in a humid chamber at 37°C with 5% CO_2.

Cell Sample Preparation

When the culture reached 70% confluence, the cells were detached using 0.025% trypsin (Life Technologies) for 5 min. Next, the cells were counted and samples were prepared at a density of 5×10^6 cells. Three samples were prepared for each cell line.

Cell sample Lyophilization

Dura Dry (FD2085B0000) and Dura Stop (TDS3BOT5000) (FTS Systems) freeze dryers were used for lyophilization. The process was controlled by the lyophilizer itself, 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 (270 min): Freezing time of the sample on the shelf at –40°C.
P (280 min): Primary freeze-drying time, with shelf at −22°C and vacuum at 200 mTorr.
S (390 min): Secondary freeze-drying time (removal of moisture residues), with shelf at +20°C and vacuum at 10 mTorr.
T (940 min): Total process time.

Preparation of Samples

High-purity deionized water (resistivity 18.2 MΩ cm) obtained using a Milli-Q® water purification system (Millipore, Bedford, MA, USA) was used throughout the study. All reagents used were of analytical grade and purchased from Sigma (St. Louis, MO, USA). HNO3 from Synth (São Paulo, SP, Brazil) was purified using a quartz sub-boiling distillation unit (Kürner, Rosenheim, Germany). All solutions were stored in polyethylene flasks. Autosampler cups, plastic bottles, and glassware were cleaned by sequential soaking in a 1.40 mol L^{-1} HNO_3 solution for 24 h and rinsing five times with ultrapure water. After this process, the materials were air-dried and stored in a class-100 laminar flow hood. Stock solutions (1,000 mg L^{-1}) were prepared from spectrographically pure reagents (Johnson and Matthey, Royston, UK) in diluted HNO_3. Analytical calibration solutions were prepared using the suitable dilution of stock solutions in a 0.028 mol L^{-1} HNO_3 solution. All operations were performed on a clean bench.

For the digestion of the samples, a Berghof Spedwave 4 microwave oven with cavity was used, with capacity for 10 vials, power of 1000 W, maximum pressure of 40 bar and temperatures of up to 230 °C. To each sample, 4 mL of HNO3, 1 mL of H_2O and 2 mL of H_2O_2 were added. The heating program used for this acid mixture is described in.

Table 1. Digestion microwave program used to decompose cell samples

Step	T (°C)	Ramp (min)	Baseline (min)	FAN
1	210	15:00	0	1
2	210	0	30:00	1

FAN: cooling step (15 min)

The determination of trace elements was carried out with 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) as the reaction gas. The sample introduction system was composed for a quartz double-pass spray chamber and a concentric nebulizer connected by Tygon® tubes to the peristaltic pump on the ICP-MS (set at 20 rpm). The ICP-MS operational conditions are described in.

Table 2. ICP-MS experimental conditions

Monitored Isotopes	
Helium Mode	^{55}Mn, ^{63}Cu, ^{66}Zn
No Gas Mode	^{59}Co, ^{56}Fe,
High Energy Helium (HEHe) Mode	^{42}Ca, ^{41}K, ^{24}Mg, ^{23}Na, ^{31}P, ^{34}S, ^{80}Se
Power	1550W
Internal Starndard	89Y 10 µg L^{-1}
Argon Flow	15 L min^{-1}
He flow on Helium Mode	5 L min^{-1}
He flow on HEHe Mode	10 L min^{-1}
Nebulizer gas flow	1.05 L min^{-1}
Spray Chamber	Scott (double pass)
Interface	Nickel
Sample Cone	1.0 mm
Skimmer Cone	0.9 mm
Helium purity	Helium > 99.99 %

Statistical Analysis

The results are presented as the mean ± standard error (SE) of single comparisons. Statistical significance was determined using the F-test and Student's t-test in Microsoft Excel. $P<0.05$ was considered statistically significant. All experiments were performed in triplicate.

Results

In this study, we used two human proximal convoluted tubule epithelial cell lines, HK-2 benign and CAK-1 malignant. As shown in Figures 1A and 1B, their morphologies are markedly different. The shape of HK-2 cells is homogenous, while Caki-1 cells show pleomorphism, one of the characteristics of cells that have undergone malignant transformation.

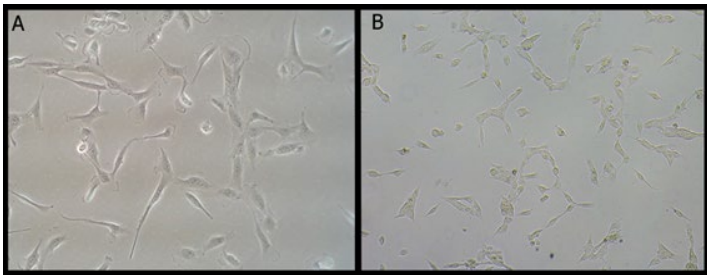


Fig. 1 Representative images of Hk-2 and Caki-1 cells in culture (20×) ECLIPSE TS100 (Nikon, Japan)

Quantitative determination was performed for 12 elements, with values expressed in micrograms per 10^6 cells. The mean profile of the elements in the HK-2 cells compared to Caki-1 cells is shown in Table 2. Almost all of the trace elements showed a significant difference between the normal kidney epithelial tubular cells and

ccRCC cells. The most marked difference was a decrease in the detection of Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn. A significant accumulation of Co was also observed in Caki-1 cells. No statistically significant differences were found for Se.

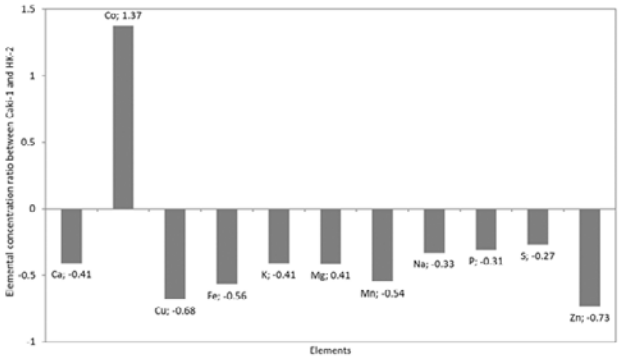
Table 3. Comparison of trace element concentrations (µg/5 × 106 cells) between normal tubular epithelial (HK-2) and RCC tumor (Caki-1) cells

Element	HK-2 cells	Caki-1 cells	P
Ca	0.770±0.15	0.456±0.08	<0.01
Co	0.0008±0.0031	0.0019±0.0005	<0.05
Cu	0.1260±0.0241	0.0403±0.0241	<0.05
Fe	0.8743±0.2483	0.3776±0.0871	<0.05
K	188.78±46.08	111.50±27.52	<0.05
Mg	7.63±0.63	4.49±0.90	< 0.01
Mn	0.039±0.0094	0.018±0.0065	<0.05
Na	1830.91± 54.96	1226.64±185.48	< 0.01
P	33.71±3.524	23.22±5.827	<0.05
S	14.50±0.79	10.60±2.27	<0.05
Se	0,0043±0,0009	0,0040±0,0019	NS
Zn	1.9015±0.1476	0.5098±0.2520	<0.01

NS Statistical difference is not significant

The concentration of the elements in Caki-1 and HK-2 cells was evaluated and compared. Consequently, the most marked variations were an increase in Co (1.37-fold change) and decreases in Zn (–0.73-fold change), Cu (–0.68-fold change), Fe (–0.56-fold change), and Mn (–0.54-fold change) (Figure 2).

Fig. 2 Elemental concentration (µg/5 × 106 cells) ratio between Caki-1 and HK-2 cells



Discussion

Malignant cells show morphological and functional characteristics that make them distinct from normal cells. Morphologically, cancerous cells are characterized by an irregular size and shape. In addition, they have an increased number of mitochondria that support processes involved in transporting ions and substances [10]. Furthermore, the function and activity of the enzyme content, and consequently the cell metabolic profile, is also distinct [11].

Our findings showed that the levels of Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn were decreased in tumor cells. These elements all play a role in the homeostasis of kidney cells. As a second messenger, Ca^{2+} regulates cellular biochemical processes; therefore, any disturbances to Ca^{2+} homeostasis can disrupt the cell cycle. Hence, the dysregulation of intracellular Ca2+ levels can affect the mechanisms of cell cycle regulation and lead to unchecked proliferation [12].

Oxidative stress, caused by elevated levels of superoxide free radicals, has been implicated in the development of RCC [13]. Superoxide dismutases (SODs) are antioxidant metalloenzymes that regulate redox homeostasis and cell signaling [14]. In humans, three isoforms have been identified: copper/zinc SOD and MnSOD2. Copper/zinc SOD (SOD1), which accounts for ~80% of total SOD protein, is constitutively present in the cytosol and mitochondrial intermembrane space of cells. On the other hand, MnSOD2 is located exclusively in the mitochondrial matrix, while Cu/Zn SOD3 is mainly extracellular [15]. Reduced levels of Cu, Zn, and Mn, which are cofactors of the antioxidant enzymes SOD-Cu/Zn and SOD-Mn, disrupt SOD activity, resulting in high levels of oxidative stress in ccRCC, contributing to tumor heterogeneity [16].

Lower concentrations of Fe in RCC tissues have been reported in previous studies [17, 18]. Fe/S clusters are found in all organelles of eukaryotic cells, where they are bound to proteins involved in various metabolic systems. Thus, decreased levels of these elements in Fe-S proteins have an impact not only in gene regulation but also in a range of diverse biological processes, including respiration, RNA modification, and DNA replication and repair [19].

Mg is the fourth most abundant cation in the body and the second most common cation in the intracellular fluid. The abundance of Mg within mammalian cells is consistent with its role in regulating tissue and cell function, where low levels of magnesium in the plasma have a deleterious effect on Lewis lung tumor metastasis [20].

Approximately 70% of Na and K are reabsorbed by proximal tubular cells via the active transporter Na^+/K^+-ATPase pump [21]. Na^+/K^+-ATPase is a membrane-bound protein that is composed of four α- and three β-subunit isoforms identified in mammalian cells. The β-subunit gene is downregulated in RCC, resulting in the deregulation of these genes in the transport of Na/K elements [22].

Co was the only element whose content was found to increase in Caki-1 cells. This element is essential to mammals in the form of cobalamin (vitamin B12), but causes deleterious effects at high levels [23]. Increased levels of intracellular Co are known to activate hypoxic signaling by stabilizing hypoxia-inducible transcription factor 1α (HIF1α), resulting in the activation of genes that support angiogenesis. Of note, angiogenesis is a feature of RCC [24].

The results of present study revealed marked differences in the concentrations of essential elements between normal and malignant kidney human cells. These findings indicate that essential elements mainly affect the metabolic profile of epithelial kidney cells. In summary, our findings provide insights into the role of essential elements in cells and open a potential new avenue for RCC target therapy.

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Conflicts of interest: The authors declare that they have no conflict of interest.

Ethics approval This article does not contain any studies with human participants or animals performed by any of the authors.

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