Magnesium intracellular content and distribution map in drug-resistant and -sensitive whole cells

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Abstract

Magnesium (Mg) plays crucial structural and regulatory roles within cells. Despite the extensive amount of data about the biochemistry of Mg, a complete picture of its regulation and cellular homeostasis is lacking. Thanks to recent improvements in third generation synchrotron X-ray sources, X-ray fluorescence microscopy (XRFM) is becoming a highly sensitive method for mapping elemental distributions in cells. XRFM maps the element content but not the concentration, which is a relevant variable in a biological context. We tackled this issue by combining XRFM with atomic force microscopy that was used to obtain morphological information of the sample. The aim of the present study was to compare the content and the distribution of Mg in drug-resistant and -sensitive tumor cell lines. Our data has shown a massive increase of Mg in LoVo drug-resistant cells. Moreover, the map of intracellular Mg showed marked differences in the pattern distribution between sensitive and resistant cells.

Introduction

Magnesium (Mg) plays crucial structural and regulatory roles within all cells. Despite the extensive amount of data about the biochemistry of Mg, a complete picture of its distribution, regulation and cellular homeostasis is lacking for both conceptual difficulties and technical limitations. Although recent efforts in applying new live imaging techniques to the field of magnesium research, an accurate characterization of Mg distribution and content in the cellular environment is still lacking. The acquisition of detailed information on intracellular processes requires high spatial resolution, quantitative data, and chemical information. In order to achieve this goal, in a recent paper[1] we have demonstrated that it is possible to merge compositional and morphological information to quantitatively derive the element concentration combining X-ray fluorescence microscopy (XRFM) with atomic force microscopy (AFM). XRFM represents an ideal technique, thanks to recent improvements in third generation synchrotron X-ray sources and in X-ray focusing optics. In particular, XRFM is a highly sensitive method for mapping elemental distributions in cells such as carbon, nitrogen, oxygen, sodium.

The aim of the present study is to compare the content and the distribution of Mg in drug-resistant and -sensitive tumor cell lines chemically fixed. The experimental model employed human colon carcinoma cell lines (LoVo) sensitive and resistant to doxorubicin, one of the anticancer drug mostly employed in the therapy of several solid tumors.

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Materials and Methods

The LoVo cells were cultured in Roswell Park Memorial Institute (RPMI) medium (Sigma Aldrich, St. Louis, MO, USA) supplemented with 10% PBS (phosphate buffered saline), 2 mM glutamine, 100 U/mL penicillin, and 100 g/mL streptomycin sulphate. At 50-80% confluency, the cells were briefly rinsed in 150 mM KCl, then fixed in ice-cold methanol/acetone 1:1 and air dried. The XRFM measurements on LoVo cells were carried out using synchrotron radiation at the TWINMIC beamLine at Elettra Institute, Trieste, Italy. XRFM can map the element content but not the concentration, which is the relevant variable in a biological context.

We tackled this issue by combining XRFM (pixel dimension 0.5 mm) with AFM (scan size 60 mm, pixel resolution 512x512, Figure 1A and 1B) that was used to obtain morphological information (thickness) of the sample analyzed.[1] The additional advantages of this novel experimental approach is to combine both high-resolution elemental and morphological information in a single cell with a sub-micrometer spatial resolution. The fluorescence spectra were analyzed with the PyMCA[2] software, and deconvoluted into their components, considering the incident photon energy of about 1.5 keV. Under these condi-

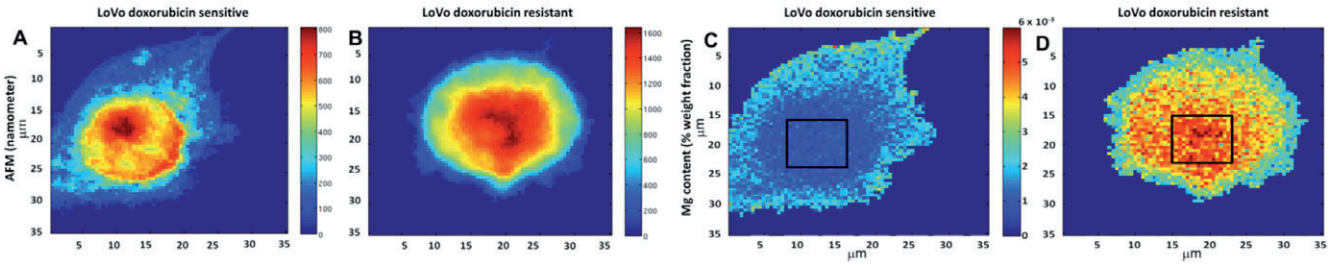

Figure 1. A,B) Atomic force microscope thickness maps and C,D) magnesium content of LoVo drug-sensitive and -resistant cells. In C,D) central squares mark the nuclei of the two cells where the mean weight fractions are calculated.

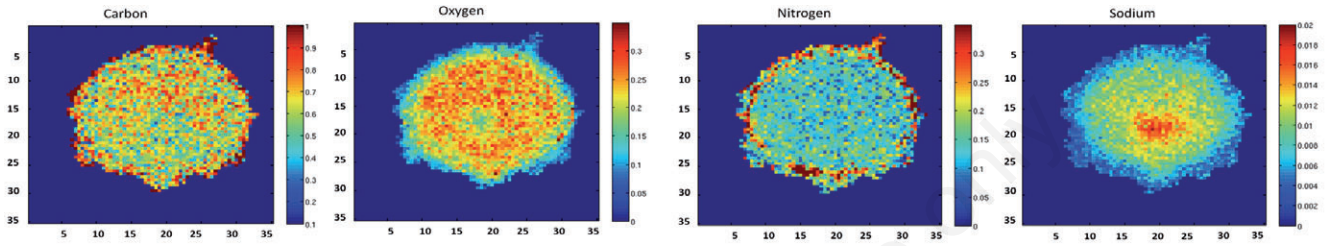

Figure 2. Content maps of carbon, oxygen, nitrogen and sodium in LoVo drug-resistant cell.

tions, we detected of carbon (C), oxygen (O), nitrogen (N), sodium (Na), and magnesium (Mg), whose fluorescence radiation is strongly affected by the absorption in the cell itself (self-absorption effect). We therefore developed a specific algorithm to correct for self-absorption,[3] and applied this procedure to obtain the actual element distribution of C, N, O, Na and Mg.

Results

Figure 1 shows the content (reported as weight fraction) and the distribution of Mg in LoVo cells, sensitive and resistant to doxorubicin. This figure clearly shows the two main outcomes of this study.

First, in the drug-resistant cell the content of Mg is massively increased. Particularly, we calculated the mean weight fraction in a region of interest (ROI) (8x8 mm) placed in the nucleus of the two cells (Figure 1C and 1D). The amount of Mg in the drug-resistant cell is 0.48%, while in the drug-sensitive one is 0.087%.

Second, the map of intracellular Mg showed a marked differences in the pattern distribution between sensitive and resistant cells.

Discussion

In this work we have demonstrated that it is possible to assess the distribution of the major cell components, together with light metal elements, with XRFM. Results of this study confirm the preliminary data recently obtained in a different cell line resistant to cis-platinum,[4] also showing that drug-sensitive and -resistant cells have a different intracellular Mg distribution. All together these findings provide further evidences that Mg plays a role in the genesis of the drug-resistant phenotype.

Conclusions

Our approach holds promise of becoming a reference methodology not only for measurements of a major cell constituent such as Mg, but also for other light elements (Figure 2). When complemented with correlative procedures aimed at characterizing sub-cellular structures and organelles, it could represent an indispensable tool for the accurate mapping of any intracellular element both in normal and pathological conditions.

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