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Application News

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Multimodal Imaging

MULTIMODAL IMAGING OF PHOTOSENSITIZERS IN 3D TUMOR CELL MODELS

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▪ Introduction

Photodynamic therapy offers an innovative and alternative cancer treatment. A photosensitive compound (photosensitizer, PS) is administered and the tumor is subsequently irradiated. The activation of the PS leads to a formation of reactive oxygen species and subsequently to cell apoptosis. The compound 5,10,15,20-tetrakis(3-hydroxy-phenyl)-porphyrin (mTHPP), and its palladium-tagged analogue mTHPP-Pd were studied in this work (Figure 1). One main challenge in the development of PS is the hydrophobic character of the compounds that hinders the tissue penetration. Additionally, the orally administered compound needs to pass through the mucus layer in the gastro-intestinal tract. Thus, the determination of the penetration depth of those compounds is of great interest.

The use of 3D tumor spheroids enables in vitro drug screening, while simulating the tumor environment better than 2D cell cultures. Here, the combination of elemental and molecular imaging by means of laser ablation-inductively coupled plasma-mass spectrometry (LA-ICP-MS) and matrix-assisted laser desorption/ionization-mass spectrometry imaging (MALDI-MSI) was used to investigate the spatial distribution and concentration of mTHPP and mTHPP-Pd in 3D tumor spheroids.

▪ Material and methods

Tumor spheroids

Stable tumor spheroids based on the human colon carcinoma cell line HT29 were obtained using the liquid overlay technique.[1] The PS mTHPP and mTHPP-Pd were synthesized according to known literature[2] and the tumor spheroids were incubated with the dissolved PS for 24 h with a final PS concentration of 5 µM.

Sample preparation

For MALDI-MSI, 14 µm thin sections of the spheroids embedded in Tissue-Tek® O.C.T. were placed on indium tin oxide-coated glass slides. The cryo-compound was removed using ammonium acetate buffer and α-cyano-4-hydroxycinnamic acid (CHCA) was sublimated onto the tissue using the iMLayer™ (20 min, 250 °C, 5·10¬2 Pa).

For LA-ICP-MS analysis, 5 µm thin tissue sections of the spheroids treated with mTHPP-Pd were placed on microscope slides to enable the detection via ICP-MS. An external calibration with matrix-matched gelatin standards was applied for quantification. To do so, 10% gelatin was spiked with aqueous palladium ICP standard solution in a concentration range from 0.1 to 100 µg/g. The standards were heated, homogenized, and cut into 5 µm thin sections, as well.

Instrumentation

For molecular imaging, the iMScope Trio was utilized. The MALDI source, equipped with a 355 nm Nd:YAG laser, was operated at atmospheric pressure with a laser pulse frequency of 1000 Hz. For each pixel, 100 laser shots were accumulated. The sample voltage was set to 3.5 kV and the detector voltage was 1.9 kV.

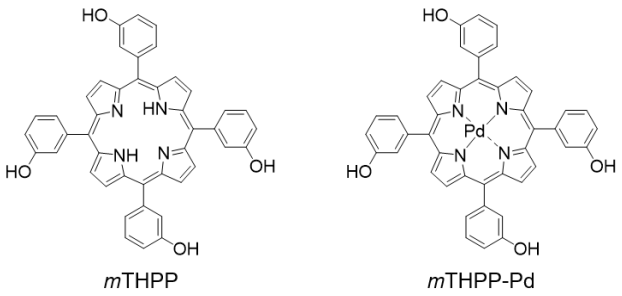

Figure 1: Chemical structures of the photosensitizer 5,10,15,20-tetrakis(3-hydroxyphe-nyl)-porphyrin (mTHPP) and the palladium(II)-tagged equivalent (mTHPP-Pd).

The laser spot size was set to 5 µm with a laser fluency of 0.068 µJ and a step size in the x- and y-dimension of 10 µm. The illustrated results were obtained in the positive ion mode from m/z 600-900. All identified molecules were confirmed by MS2 experiments using argon as collision gas and an isolation and collision-induced dissociation time of 20 and 30 ms, respectively.

For elemental bioimaging, LA-ICP-MS was applied. Here, the ICPMS-2030 quadrupole mass spectrometer was coupled to a laser ablation system LSX-213 G2+ (Teledyne CETAC). The system was equipped with a Nd-YAG laser operating at 213 nm and the ablation cell was flushed with 0.8 l/min helium.

The laser was operated at a frequency of 20 Hz with a spot size of 7 µm and a scan speed of 21 µm/s. HNO3 (2% w/v) was introduced with a nebulizer gas (argon) flow of 0.45 l/min for improved plasma stability. The ICP-MS was equipped with nickel sampler and skimmer cones and was operated in the collision mode with helium as collision gas (6 ml/min). The isotope 31P was detected with an integration time of 85 ms, whereas the integration time for the isotopes 105Pd and 106Pd was 100 ms. The plasma settings were as following: plasma power 1.2 kW, torch depth 5 mm, plasma gas 9.0 l/min.

▪ Results

The MALDI-MS images of a tumor spheroid treated with mTHPP are shown in Figure 2. In the microscopic image, an almost spherical tissue section with a diameter of approx. 550 µm can be seen. The phosphatidyl-cholines PC 32:1, PC 34:1, and PC 36:1 are well suited to visualize the tumor tissue and exhibit a correlating distribution. The distribution map of mTHPP shows a ring-shaped distribution, which can be precisely correlated with the outer cell layer of the tumor spheroid as it is shown in the overlay of the microscopic image with the MALDI-MS data.

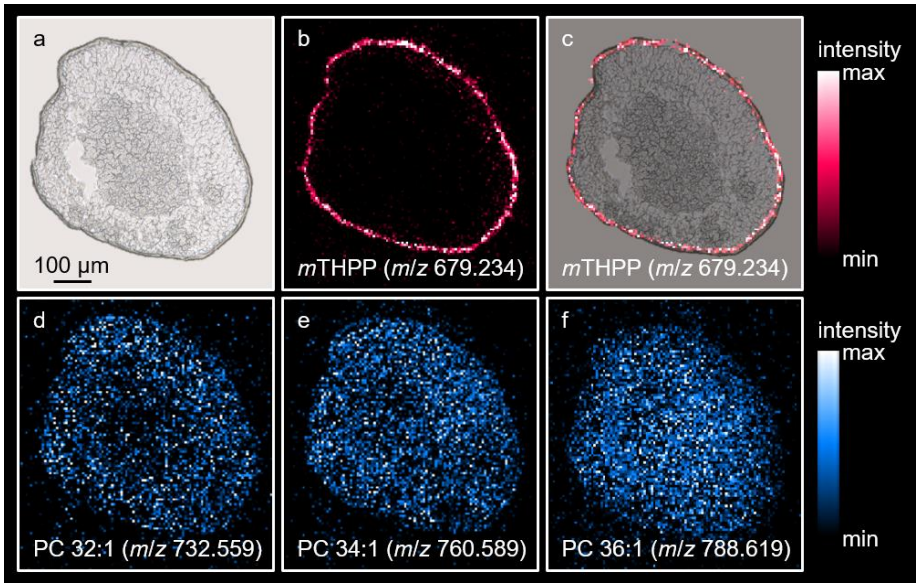

Figure 2: MALDI-MS images of a spheroid treated with dissolved mTHPP. The microscopic image (a), the distribution of mTHPP (b), and an overlay of both (c) are shown. In addition, the TIC-normalized distribution of the three phospholipids PC 32:1 (d), PC 34:1 (e), and PC 36:1 (f) are depicted. All molecules were detected as [M+H]+ and are illustrated in a mass window of ±0.05.

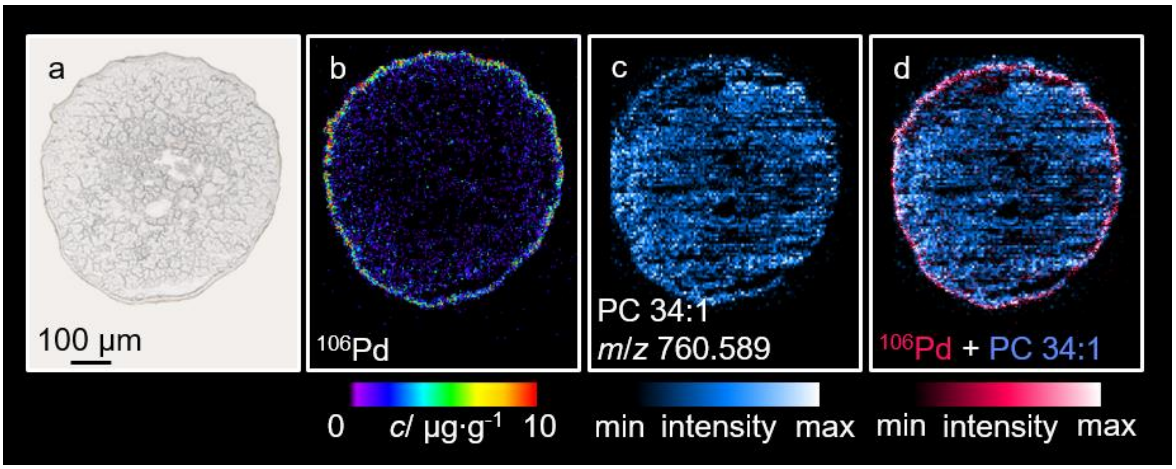

Figure 3: Combined elemental and molecular imaging on the same thin section of a HT29 tumor spheroid treated with mTHPP-Pd. The microscopic image (a) is shown next to the LA-ICP-MS image of 106Pd (b), the MALDI-MS image of PC 34:1 (C), and an overlay of both images.

The PS is distributed homogeneously inside the outer layer and not around the spheroid, tough it does not penetrate deeper into the tissue. Nevertheless, the MALDI-MS experiments revealed that the PS can be detected as intact molecule without substantial decomposition during the sample preparation.

In order to combine the advantages of molecular information and straight-forward quantification, a tumor spheroid thin section was first investigated using MALDI-MS, and afterwards by means of LA-ICP-MS. The results for a spheroid incubated with mTHPP-Pd is shown in Figure 3. Since the metal-tagged PS is needed for ICP-MS analysis, only spheroids treated with this compound could be investigated. Conversely, this modification of the molecule could no longer be detected using MALDI-MS. Due to the loading with palladium, the preferred protonation sites of the molecule are unavailable impairing the detection. However, MALDI-MS can be used to identify phospholipids. Palladium concentrations up to 10 µg/g with an average of 1.9 µg/g were detected. In addition, the distribution of the PS is identical with the previous findings and shows a localization only in the outer cell layers. Besides this, the phospholipids described before could be detected. Exemplarily, the distribution of PC 34:1 is shown and can be used to assign the tissue structure. An overlay of images from both methods can be used to visualize the distribution of mTHPP-Pd around the phospholipid-rich 3D tumor cell model.

■ Conclusion

In conclusion, the employed imaging techniques present complementary methods to study of the penetration depth of PS in tumor spheroids as new therapeutic alternative for the cancer treatment. The direct combination of molecular and elemental bioimaging can be a useful tool, combining the advantages of both, MALDI-MS and LA-ICP-MS imaging. With this approach, the structural identification of the substance of interest by means of MS2, as well as the quantification using matrix-matched standards was realized.

■ References

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[2] A.-C. Niehoff, A. Moosmann, J. Söbbing, et al., Metallomics. 6 (2014) 77–81.

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