

Proteomic session

Identification of Protein Targets involved in Photodynamic Therapy (PDT) by complementary approaches

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In Photodynamic Therapy (PDT), a light-sensitive compound, Foscan[®] is intravenously administrated. This photosensitizer, which is preferentially localized in tumor tissue is activated by irradiation at an appropriate wavelength. Free radicals and singlet oxygen are then produced and lead to cell death by apoptosis and/or necrosis.

In order to better understand PDT mechanisms in cellular media, a proteomic approach has been undertaken. Relying on the Bottom-Up strategy, MALDI-FTICR mass spectrometry allows oxidative protein modifications to be identified. Results obtain by this technique have been compared to MALDI-TOF/TOF results on the same protein batches.

After comparison of proteins distribution corresponding to treated and untreated HT29 cells, 60 modified proteins were evidenced and identified using our developed proteomic method. The identification was carried out on MALDI-FTICRMS data (high resolution and high mass accuracy) and validated by two techniques MALDI-TOF-TOFMS data (middle resolution and middle mass accuracy) and nanoLC-MS/MS using a Target-Decoy strategy. This proteomic approach has also allowed to classify the modified proteins according to two criteria: protein function and intracellular localization. For example: protein disulfide-isomerase A3 (Score: 111) which are known to have Chaperone Functions and are localized in Endoplasmic Reticulum, appears to be under expressed when cells are treated by PDT.

PDIA3 sequence coverage is 41 % for each technique. 26% of the sequence is common to both techniques, 16% of the sequence are specifically covered by MALDI-FTICRMS and 16 % specifically by MALDI-TOF-TOFMS. Thus, the total coverage of the protein sequence using the sum peak lists of the two techniques is reaching 58%. This example is well illustrating complementary between these two MALDI-MS techniques. Even if MALDI-TOF-TOF is recognized to be the reference for the proteomic analysis, MALDI-FTICRMS can confirm the attribution in some cases and allows increasing the level of assignment confidence in other cases. To understand this complementary, we tested the influence on protein identification considering various matrices (cold "DHB" and hot "HCCA" matrix) and both mass spectrometry techniques.