Combining mass spectrometry imaging and in vivo luminescence imaging to study the biomolecular profile of relapsed diffuse large B-cell lymphoma

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Abstract

Diffuse large B-cell lymphoma (DLBCL) is the most common B-cell non-Hodgkin’s lymphoma [1]. Currently, R-CHOP, a combination of immunotherapy (R; rituximab) and chemotherapy (CHOP; cyclophosphamide, doxorubicin, vincristine and prednisone), remains the most commonly used regimen for newly diagnosed DLBCLs. DLBCL is an aggressive disease and up to one-third of patients ultimately become refractory to initial therapy or relapse after treatment [2]. The high mortality rate highlights the urgent need for novel therapeutic approaches based upon selective molecular targets.

Changes in the chemical composition during tumoral development and treatment response were studied with a combination of in vivo DLBCL xenograft models and mass spectrometry imaging (MSI), providing information regarding analyte composition and molecular distributions of therapy resistant and sensitive areas.

Luminescent human DLBCL cell lines were xenografted into immune-deficient mice. Tumoral development was followed and quantified based on their luminescent signal using an in vivo imaging system (IVIS) Lumina II (Caliper Life Science). A RapifleX MALDI-TOF Tissuetyper instrument (Bruker Daltonik GmbH, Bremen, Germany) was used for high speed imaging at a spatial resolution of 100 µm. An Orbitrap Elite Hybrid Ion Trap Mass Spectrometer (Thermo Fisher Scientific, Bremen, Germany) was additionally used to confirm the molecular identity of the lipids and metabolites. After MSI analysis, the tissues were H&E stained for co-registration with histology. FlexImaging v4.1 (Bruker Daltonik GmbH, Bremen, Germany) and SCiLS Lab 2016b (SCiLS GmbH, Bremen, Germany) were used to process the data acquired with the Bruker RapifleX. Thermo Xcalibur 3.0.63 (Thermo Fisher Scientific, Bremen, Germany) was used to analyze the orbitrap data. Principal component analysis (PCA), hierarchical clustering analysis (HCA) and linear discriminant analysis (LDA) were performed using an in-house built ChemomeTricks toolbox for MATLAB version 2014a (The MathWorks, Natick, USA).

Differences between the lipidomic profiles of the untreated and relapsed DLBCL have been found, revealing distinctive signatures of lipids in R-CHOP resistant lymphoma cells. Intra-tumor heterogeneity was especially relevant in the relapsed tumors. In particular, necrotic areas were only present in some of the relapsed tumors. The score plot projections show that it is possible to discriminate between both conditions based on the metabolite data. Lipids are also ionized and thus contributing to this discrimination. Therefore, a more specific protocol for metabolite extraction will give more information on the metabolic differences. Further research is needed to validate these differences and to investigate the biology causing these changes.