A combined laser microdissection and proteomic analysis method for identification of robust biomarkers in oncology.

Elodie HENRIET 1,6, Aya ABOU HAMMOUD 1,6, Sylvaine DI TOMMASO 6, Jean-William DUPUY 2, Benjamin DARTIGUES 2, Zakaria EZZOUKRY 1, Nathalie DUGOT-SENANT 3, Thierry LESTE-LASSERRE 4, Nestor PALLARES-LUPON 1, Macha NIKOLSKI 2, Brigitte LE BAIL 1,6, Jean-Frédéric BLANC 1,6, Charles BALABAUD 1, Paulette BIOULAC-SAGE 1,6, Anne-Aurélie RAYMOND 1,6,6, Frédéric SALTEL 1,6,6,6,6, &

1 Bordeaux Research in Translational Oncology
2 Centre de génomique fonctionnelle de Bordeaux
3 TBMCore
4 Neurocentre Magendie
5 CHU de Bordeaux
6 Oncoprot http://www.tbmcore.u-bordeaux.fr/oncoprot/
&. * The authors contributed to equal work

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Purpose: (Times New Roman, font12)
Since several years, research efforts concentrated on the identification of genomic abnormalities in tumors offering the prospect of personalized treatments and thus a better management of patients. However, proteins expression is the downstream result of these combined genomic anomalies in tumors and is essential for a better understanding of the mechanisms of cancer initiation, tumoral progression, metastatic scattering and to identify new biomarkers and pharmacological targets.

Experimental Design: (Times New Roman, font12)
Mass spectrometry is the method of choice to identify, characterize and quantify the proteins in a complex sample. The Oncoprot platform (http://www.tbmcore.u-bordeaux.fr/oncoprot/) developed a method combining laser microdissection and mass spectrometry analysis to compare the proteomic profiles of tumors. This procedure has been optimized for the study of FFPE tissue sections even on small material obtained from biopsy.

Results: (Times New Roman, font12)
We made the proof of concept on Hepatocellular adenomas (HCA), rare benign tumors that constitute a heterogeneous entity, divided into several groups based on patho-molecular features ((1) HCA with inactivating mutations of HNF1A, (2) Inflammatory HCA with diverse mutations leading to the activation of STAT3, (3) HCA with activating beta-catenin. In the case of unclassified HCA (UHCA), which were identified by default, a high risk of bleeding remained a clinical issue. The objective of this study was to explore UHCA proteome in the purpose of identifying specific biomarkers. We compared tumoral and non-tumoral proteins expression levels in HCA, H-HCA, IHCA, b-HCA, and UHCA. Using the Oncoprot’s process, we searched for proteins specifically deregulated in UHCA. First, we demonstrated that proteomic profiles allow us to discriminate between known HCA subtypes by the identification of classical biomarkers of each HCA subgroup. We revealed specific upregulation of the synthesis of the arginine pathway associated with an overexpression of argininosuccinate synthase (ASS1) and argininosuccinate lyase (ASL) in UHCA. Moreover, arginine is the substrate for nitric oxide synthesis, a factor involved in vascular permeability. ASS1 immunohistochemistry identified all the UHCA, of which 64.7% presented clinical bleeding manifestations.

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**Conclusion:** In conclusion, we demonstrated the power of mass spectrometry coupled with laser microdissection to identify tumoral signatures and robust biomarkers. We can now answer to other clinical needs to propose new biomarkers for diagnosis, prognosis and to predict the response to treatments.

**References:**