Metabolomics implicates dimethylsphingosine in neuropathic pain

Researchers use metabolomics to identify novel molecules that could form the basis of drug targets across a number of diseases.

Neuropathic pain is a condition that causes major chronic pain in millions of people worldwide. The reasons that cause this pain to persist remain unclear, and treatment has been inhibited by a lack of understanding of the chemical basis of this pain. Researchers have recently implicated dimethylsphingosine (DMS) as a molecule that is associated with inducing neuropathic pain. The group, led by Garry Patti from Washington University, St. Louis (MI, USA), used metabolomics to implicate DMS.

The use of metabolomics to track small molecules such as amino acids, sugars and vitamins allows an alternative approach to determining the differences exhibited between healthy and diseased cells. Patti explained this further: "These are the molecules that are actually being transformed during cellular activity, and tracking them provides more direct information on what is happening at a biochemical level."

Gary Siuzdak from the Scripps Research Center for Metabolomics (CA, USA) explained the strategy used in this set of experiments: "The idea was to apply metabolomic analysis to understand the biochemical basis of the neuropathic pain condition and reveal potential therapeutic targets. We call this approach therapeutic metabolomics." The group used rats as models for neuropathic pain. Samples were taken from the blood plasma, spinal cord and injured tibial leg nerve. These were analyzed and the metabolites compared with healthy controls.

The potential importance of metabolomics as a novel approach to solving vexing biological issues was highlighted by Siuzdak: "We're very excited about this therapeutic metabolomics approach. In fact, we are already involved in several other projects in which metabolites are giving us a direct indication of disease biochemistry and potential treatments." Speaking to *Expert Review of Proteomics*, Patti further commented that: "Our study demonstrates the biological and therapeutic insight that can be gained from using untargeted metabolomics not only to unbiased profile known pathways, but also to identify physiologically important metabolites that have yet to be characterized."

Referring back to the issues concerning neuropathic pain, Patti explained that: "This is the first characterization and quantitation of DMS as a naturally occurring compound. We think that this is a big step forward in understanding and treating neuropathic pain, and also a solid demonstration of the power of metabolomics." Research is now likely to shift towards looking for the enzymes involved in synthesizing DMS along with potential drug targets.

– Written by Andreas Hadjivasiliou

Source: Patti GJ, Yanes O, Shriver L *et al.* Metabolomics implicates altered sphingolipids in chronic pain of neuropathic origin. *Nat. Chem. Biol.* 8(3), 232–234 (2012).

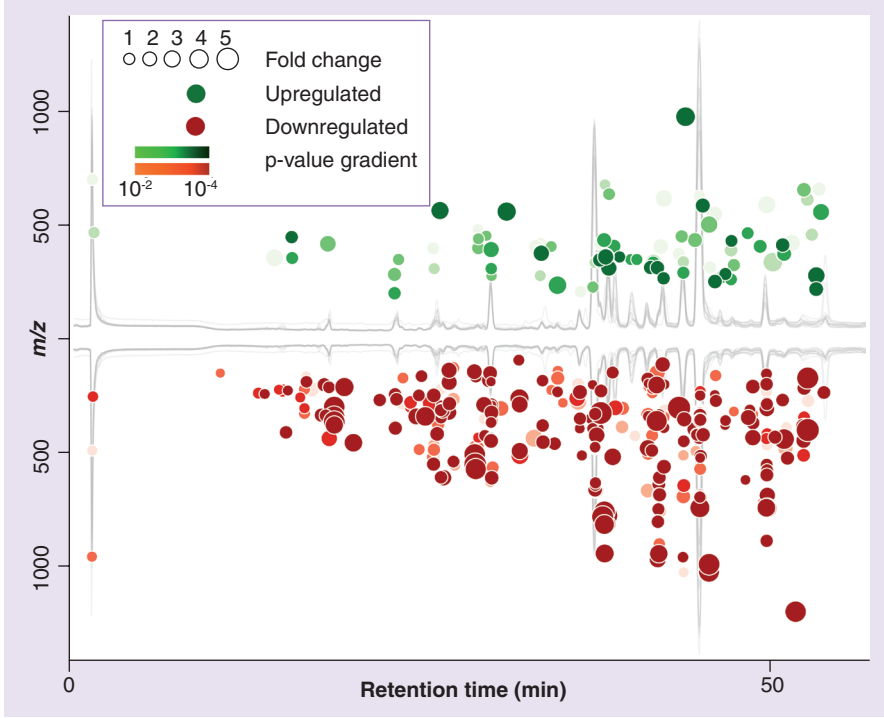

Figure. The 'mirror plot' represents a new way of visualizing differential regulation derived from mass spectrometry data. In this representation of the untargeted metabolomics liquid chromatography/mass spectrometry experiments, the data ultimately revealed that sphingomyelin–ceramide metabolism is altered in the dorsal horn of rats with neuropathic pain.
Figure provided by Gary Patti, Ralf Tautenhahn and Gary Siuzdak.

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New study highlights the potential of SISCAPA MALDI-TOF mass spectrometry for biomarker validation and clinical research

Results of the robustness and throughput of MALDI-TOF compared with conventional nano-liquid chromatography–mass spectometry technology may allow currently impractical large-scale verification studies of protein biomarkers.

A study published in the *Journal of Proteome Research* has shown that 'stable isotope standards and capture by anti-peptide antibodies' (SISCAPA) MALDI-TOF mass spectrometry (MS) is capable of providing a high degree of precision for the quantitation of proteins of interest, such as high-value protein biomarkers. These results are the product of an ongoing effort between Bruker Corporation (Bremen, Germany) and SISCAPA Assay Technologies, Inc. (Washington DC, USA) to develop MALDI-TOF as a high precision MS detection method for SISCAPA assays.

The published results demonstrate that the use of internal isotopically labeled peptide standards in the SISCAPA assay yields very precise relative quantitation of peptides (coefficients of variation of 1–2%), which compares favorably with the results obtained to date by the best conventional nano-liquid chromatography (LC)–MS workflows. As a result of the removal of complex and time-consuming procedures, nano-flow LC–MS separation significantly decreases the complexity of sample handling and sample introduction. Together with advances in automated preparation of MALDI target plates, large-scale biomarker verification studies that are currently considered impractical will be more feasible.

Leigh Anderson, CEO and founder of SISCAPA Assay Technologies, and inventor of SISCAPA, commented, "Our joint paper demonstrates the very high precision of MALDI-TOF for peptide quantitation using internal standards, and establishes a solid basis for further exploring the utility of SISCAPA MALDI-TOF in biomarker verification and preclinical research. As a company devoted to the development and application of SISCAPA assays, we are excited that the addition of this robust and easy MALDI-TOF workflow to SISCAPA will not only increase the productivity of labs using SISCAPA, but also increase the adoption of SISCAPA assays by the biomarker research community."

– Written by Paolo Reveglia

Source: Anderson NL, Razavi M, Pearson TW, Kruppa G, Paape R, Suckau D. Precision of heavy–light peptide ratios measured by MALDI-TOF mass spectrometry. *J. Proteome Res.* doi:10.1021/pr201092v (2012) (Epub ahead of print).

Study reveals regulatory mechanism implicated in glioblastoma

Glioblastoma is one of the most aggressive cancers and the most common brain cancer. Research carried out at the Vall d'Hebron University Hospital (Barcelona, Spain) has reported that the deubiquitinating enzyme ubiquitin-specific peptidase 15 (USP15) stabilizes TGF-β, which is widely considered to be a potential therapeutic target.

The initial identification of USP15 was achieved using a functional RNAi screen. The group, led by Joan Seoane from the Vall d'Hebron University Hospital, then generated a patient-derived orthotopic mouse model suffering from glioblastoma. Further results that were reported by the group indicated that depletion of USP15 resulted in a decrease in the level of tumor generation in patient-derived glioma-initiating cells. This was also attributed to repressed TGF-β signaling. The groups results also indicated that USP15 is an activator of the TGF-β chain reaction. This was considered important, as in tumors USP15 is overexpressed, resulting in aberrant TGF-β activation. USP15 binds to a complex, deubiquitinates it and then stabilizes a TGF-β receptor causing an elevated TGF-β signal. Seoane neatly summarized the main results, stating: "When we inhibited USP15 in a real model of human glioblastoma, TGF-β activity decreased and the tumor did not develop. USP15 regulates tumor progression and is critical in cancer." Another aspect reported in this work was the impact of overexpression of the *USP15* gene due to mutations. This has been shown to be involved in a number of other cancers, such as breast cancer.

The implications arising from these results were also discussed by Seoane, who commented that: "Enzymes in general, particularly deubiquitinating enzymes such as USP15, can easily be deactivated and are therefore good therapeutic targets. Our results, generated thanks to the funding received from the Spanish Association Against Cancer, show exciting new promise in improved treatment of cancer patients."

– Written by Andreas Hadjivasiliou

Source: Eichhorn PJ, Rodón L, Gonzàlez-Juncà A *et al.* USP15 stabilizes TGF-β receptor I and promotes oncogenesis through the activation of TGF-β signaling in glioblastoma. *Nat. Med.* doi:10.1038/nm.2619 (2012) (Epub ahead of print).

Proteomics used to identify potential biomarkers for dengue hemorrhagic fever

Researchers at the University of Texas Medical Branch (TX, USA) have recently published a pair of papers detailing improved methods to detect dengue hemorrhagic fever (DHF). The papers, published recently in *The American Journal of Tropical Medicine and Hygiene* and *Clinical and Translational Science*, detail the efforts by the group to improve early detection systems of DHF using modeling and proteomics. Currently, patient outcomes when treated early for DHF are considerably more favorable than late-stage treatment.

Lead author Allan Brasier, Director of the University of Texas Medical Branch Institute for Translational Sciences, explained the background of the research: "We have long known that dengue has many manifestations, from asymptomatic to a flu-like state to a life-threatening condition. If we could figure out early a patient's susceptibility to the deadly form, we could save thousands of lives." The group used modeling and proteomics to achieve this goal. Brasier commented: "Until now, biomarkers of the disease have proved elusive. But proteomics technologies are changing the landscape and these studies are the first step toward a personalized approach to treating dengue infection."

Speaking to *Expert Review of Proteomics*, Brasier neatly encapsulated the main thrust of the work and hinted at further possible applications: "These two studies demonstrate the feasibility of using combinations of lab measurements, blood counts and proteins, to develop a personalized medicine approach for risk assessment of acute infectious disease outcome. Our findings further show how best to combine proteomics measurements into predictive modeling using nonparametric modeling. The NIAID Clinical Proteomics Center is using these same approaches for multivariate identification of risk for other infectious diseases, including *Helicobacter pylori* infection, invasive aspergillosis and Chagas disease."

Brasier summarized the future potential of the published work: "We have proved it is feasible to identify predictive proteins associated with DHF. If future research bears out these candidate proteins as firm predictors of DHF, doctors can act early to save lives – the highest hope for personalized medicine." Further detail on ongoing efforts can be found at the Clinical Proteomics Center for Infectious Disease and Biodefense website.

– Written by Andreas Hadjivasiliou

Sources: Brasier AR, Ju H, Garcia J *et al.* A three-component biomarker panel for prediction of dengue hemorrhagic fever. *Am. J. Trop. Med. Hyg.* 6(2), 341–348 (2012); Brasier AR, Garcia J, Wiktorowicz JE *et al.* Discovery proteomics and nonparametric modeling pipeline in the development of a candidate biomarker panel for dengue hemorrhagic fever. *Clin. Trans. Sci.* doi:10.1111/j.1752-8062.2011.00377.x (2012) (Epub ahead of print); Clinical Proteomics Center for Infectious Disease and Biodefense: https://bioinfo.utmb.edu/CPC

Study indicates which prion protein is most toxic

Prion diseases are neurodegenerative conditions characterized by misfolded proteins that cause severe neuronal dysfunction and death. Researchers at the Scripps Research Institute (FL, USA) have claimed to have identified the most toxic form of prion protein (PrP) from an animal study.

The group, led by Corinne Lasmézas from the Scripps Research Institute, sought to identify without any prior preconceptions or assumptions the most toxic form of the Prp protein as a potential target for future treatment. Previous work has indicated that the toxic forms of the PrP protein may differ from infectious forms. The group identified toxic forms of the PrP protein using size fractionation, dilution refolding and systematic biological testing of all fractions. The most toxic protein was identified as being a monomeric highly α-helical form of PrP. Experiments in animal models revealed that this PrP was responsible for autophagy and apoptosis, as well as a molecular signature similar to that observed in prion-infected animals. Lasmézas commented on the significance of the work, stating: "By identifying a single molecule as the most toxic species of prion proteins, we have opened a new chapter in understanding how prion-induced neurodegeneration occurs." Considering the behavior of the toxic PrP in relation to the neurodegenerative process, Lasmézas went further, reflecting: "Now we have a powerful tool to explore the mechanisms of neurodegeneration."

The potential to apply the results and methodology used in this work was another feature of the results of the study. Lasmézas speculated that: "Until now, it was thought that oligomers of proteins are toxic in all these diseases. Since we found for the first time that an abnormally folded monomer is highly toxic, it opens up the possibility that this might be true also for some other protein-misfolding diseases as well." The results of this study have been described as promising for other similar diseases such as Alzheimer's, Parkinson's and Creutzfeldt–Jakob disease as researchers look to better understand how these diseases spread and attempt to identify drug targets.

– Written by Andreas Hadjivasiliou

Source: Zhou M, Ottenberg G, Sferrazza GF, Lasmézas CI. Highly neurotoxic monomeric α-helical prion protein. *Proc. Natl Acad. Sci. USA* 109(8), 3113–3118 (2012).

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