Pressure BioSciences Inc.

PCT NEWS

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Volume 9, Issue 6 June 2015

Better Classification of Prostate Cancer Tissue Biopsy Samples Enabled by Pressure BioSciences' PCT Platform

Results of Study Led by Renowned Swiss Research Team Cites PCT as a Key Component in the Identification of New Protein Biomarker Candidates for More Precise Classification of Prostate Cancer

Links to PCT-related Posters Presented at ASMS 2015 in this Issue

Better Classification of Prostate Cancer Tissue Biopsy Samples Enabled by Pressure BioSciences' PCT Platform

Results of Study Led by Renowned Swiss Research Team Cites PCT as a Key Component in the Identification of New Protein Biomarker Candidates for More Precise Classification of Prostate Cancer

South Easton, MA, June 29, 2015 – Pressure BioSciences, Inc. (OTCQB: PBIO) ("PBI" and the "Company"), a leader in the development and sale of broadly enabling, pressure cycling technology ("PCT")-based sample preparation solutions to the worldwide life sciences industry, today announced that Tiannan Guo, MD Ph.D. of the Institute of Molecular Systems Biology, ETH Zurich, presented data on an improved method for the proteomic profiling and classification of prostate cancer tissue biopsy samples at the recent annual conference of the American Society for Mass Spectrometry.

Dr. Guo and colleagues combined the Company's enhanced Barocycler NEP2320 instrument and MicroPestle consumable ("PCT-HD") with AB Sciex's SWATH-MS mass spectrometric system (together, "PCT-SWATH") to permit what they characterize as the high throughput, reproducible, quantitative profiling of proteins in biopsy tissues. The authors concluded that PCT-SWATH (i) separated previously histologically-indistinguishable tissue biopsy samples, and (ii) identified new protein biomarker candidates for more precise classification of prostate cancer. The authors indicated their results await further validation in independent studies.

Dr. Nate Lawrence, Vice President of Marketing and Sales for PBI, said: "Patients with prostate cancer are generally classified into three groups based on histological results: low-grade, intermediate-grade, and high-grade. Patients in the intermediate-grade group most often have mixed clinical outcomes. Consequently, prostate cancer patients in this group need to be better profiled and differentiated. This should enable more precise classification, which should then lead to better prognosis and treatment. Unfortunately, the authors believe that better classification is not achievable with established diagnostic procedures."

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UPCOMING EVENTS

Pressure BioSciences, Inc. (PBI) to showcase its new cutting-edge Barozyme HT48 high throughput instrument for enhanced enzymatic digestion of proteins and PCT-HD for both protein extraction and digestion from biopsy-sized tissue samples at the HUPO 14th Annual World Congress (HUPO 2015), September 27 – 30, 2015, Vancouver Convention Center, Vancouver, British Columbia, Canada

Excerpted from Poster Presented at 2015 ASMS

Stratification of Histologically Indistinguishable Prostate Cancer Tissue Biopsy Samples Using Quantitative Proteotypes Obtained by PCT-SWATH

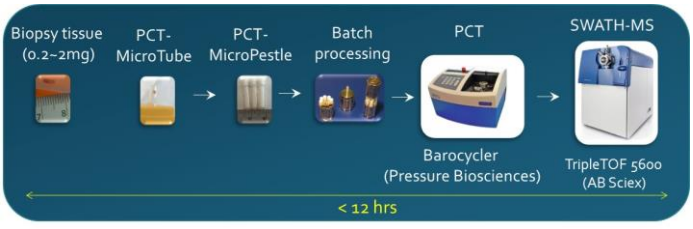
Tiannan Guo[1]; Li Li[2]; Ulrich Wagner[3]; Qing Zhong[3], Christine Fritz[3]; Christian Fankhauser[3]; Cedric Poyet[3]; Rebecca Hartmann[3]; Andreas Beyer[2]; Peter Wild[3]; Ruedi Aebersold[1, 4]; [1]Dep Biol,Inst Mol Sys Biol, ETH Zurich, Zurich, Switzerland; [2]CECAD, University of Cologne, Cologne, Germany; [3]Institute of Surgical Pathology, University Hospit, Zürich, Switzerland; [4]Faculty of Science, University of Zurich, Zurich, Switzerland.

INTRODUCTION

Prostate cancer (PC) patients are conventionally classified into three histological groups, *i.e.* low-grade, intermediate-grade and high-grade. The intermediate-grade group contains patients of mixed clinical outcomes that cannot be better classified using established diagnostic routines.

Here, we hypothesize that the quantitative proteomic analysis of tissue biopsy samples could be used to stratify intermediate-grade PC patients.

To obtain accurate quantitative proteotypes (the acute state of the proteome) of tissue biopsy samples, we used the recently developed combination of PCT (pressure cycling technology) and SWATH-MS (reference 1), which permits relative quantification of thousands of proteins from tissue biopsies with reproducibility and quantitative accuracy comparable to SRM, the gold standard quantitative MS method.



RESULTS

We analyzed 79 out of the 105 PCT-processed biopsy samples using data-dependent acquisition (DDA) mode in TripleTOF, and build a SWATH assay library for prostate tissues (reference 2).

The library contains 70,981 precursors from 59,017 proteotypic peptides in 6,686 SwissProt proteins, of which 4,991 proteins contain at least 2 peptides. 1695 proteins contain only one peptide.

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Dr. Lawrence continued: "Using PCT-SWATH, the investigators obtained a level of reproducibility and quantitative accuracy comparable to SRM, the "gold standard" quantitative mass spectrometric method. However, unlike SRM, which often results in the maximal quantification of a few hundred proteins per sample, PCT-SWATH resulted in the quantification of thousands of proteins, with unprecedented speed and precision, thus offering the potential for better classification of prostate cancer patients."

Mr. Richard T. Schumacher, President and CEO of PBI, stated: "According to the American Cancer Society ("ACS"), prostate cancer is the second most common cancer and the second leading cause of cancer death in American men. In 2015, the ACS estimates there will be approximately 220,800 new cases of prostate cancer, with about 27,540 deaths. Current statistics indicate that about one man in thirty-eight will die of prostate cancer. Consequently, laboratory tools that can better diagnose and correctly classify the grade of prostate cancer are vitally needed. We are pleased that one of the top Key Opinion Leader laboratories in the world has adopted PCT as a routine method for mass spectrometry sample preparation. We are further pleased that they believe PCT-SWATH offers the potential for wide applications in personalized medicine and that this method may ultimately achieve adoption in the clinical laboratory setting."

About PCT-HD and PCT-SWATH

The PCT-HD System combines two of the Company's unique products: the recently released, patent-pending MicroPestle consumable with an enhanced Barocycler NEP2320 instrument. This combination enables faster, less cumbersome and higher quality homogenization, extraction, and digestion of proteins. PCT-HD was developed by the Company's scientists and engineers in collaboration with Professor Ruedi Aebersold and Dr. Tiannan Guo of the Institute of Molecular Systems Biology, ETH Zurich, and the University of Zurich, both in Zurich, Switzerland. Drs. Aebersold and Guo have recently combined PCT-HD with AB Sciex's SWATH-Mass Spectrometry – calling the resulting method "PCT-SWATH".

About Pressure BioSciences, Inc.

Pressure BioSciences, Inc. ("PBI") (OTCQB: PBIO) develops, markets, and sells proprietary laboratory instrumentation and associated consumables to the estimated $6 billion life sciences sample preparation market. Our products are based on the unique properties of both constant (i.e., static) and alternating (i.e., pressure cycling technology, or PCT) hydrostatic pressure. PCT is a patented enabling technology platform that uses alternating cycles of hydrostatic pressure between ambient and ultra-high levels to safely and reproducibly control bio-molecular interactions. To date, we have installed over 250 PCT systems in approximately 160 sites worldwide. There are over 100 publications citing the advantages of the PCT platform over competitive methods, many from key opinion leaders. Our primary application development and sales efforts are in the biomarker discovery and forensics areas. Customers also use our products in other areas, such as drug discovery & design, bio-therapeutics characterization, soil & plant biology, vaccine development, histology, and forensic applications.

Forward Looking Statements

Statements contained in this press release regarding PBI's intentions, hopes, beliefs, expectations, or predictions of the future are "forward-looking" statements within the meaning of the Private Securities Litigation Reform Act of 1995. These statements are based upon the Company's current expectations, forecasts, and assumptions that are subject to risks, uncertainties, and other factors that could cause actual outcomes and results to differ materially from those indicated by these forward-looking statements. These risks, uncertainties, and other factors include, but are not limited to, the risks and uncertainties discussed under the heading "Risk Factors" in the Company's Annual Report on Form 10-K for the year ended December 31, 2014, and other reports filed by the Company from time to time with the SEC. The Company undertakes no obligation to update any of the information included in this release, except as otherwise required by law.

For more information about PBI and this press release, please click on the following website link:

http://www.pressurebiosciences.com

Please visit us on Facebook, LinkedIn, and Twitter

Stratification of Histologically Indistinguishable Prostate Cancer Tissue Biopsy Samples Using Quantitative Proteotypes Obtained by PCT-SWATH: **Continued from Page 1, Column 2**

CONCLUSIONS

1. PCT-SWATH permits high-throughput, reproducible quantitative proteomic profiling of biopsy tissues.
2. The thus obtained proteotypes separated histologically indistinguishable tissue biopsy samples.
3. New protein biomarkers candidates for precise stratification of prostate cancer were identified, awaiting for further validation in independent cohorts.

Large-Scale Quantitative Proteomic Analysis Identifies Multiple Pathways in COPD-Associated Lung Cancer

B. Sandri, PhD[1], A. Limper, MD[2], P. Jagtap, PhD[4], Y. Ping, MD[2], C. Murie, PhD[3], O. Larsson, PhD[3], P. Bitterman, MD[1], T. Griffin, PhD[4], L. Higgins, PhD[4], T. Markowski, BS[4], C. Wendt, MD[1] [1]University of Minnesota Minneapolis, MN/US, [2]Mayo Clinic Rochester, MN/US, [3]Karolinska Institute Solna/SE, [4]Center for Mass Spectrometry and Proteomics, St. Paul, MN/US

Introduction

1. Chronic Obstructive Pulmonary Disease (COPD) and Lung Cancer affect 25 million Americans.
2. Clinical reports support COPD as a risk factor for lung cancer, independent of smoking.
3. The underlying mechanisms that predispose COPD patients to lung cancer remains unknown.
4. Developed comprehensive analytical workflows capable of data normalization across 13 iTRAQ runs.

Goal

Identify molecular pathways in the etiology of lung cancer associated with COPD using wide-scale 8-plex iTRAQ with MS/MS along with a highly-customized workflow and data processing using the GalaxyP platform.

Tissue processing:

Lung tissue (100 mg) washed in a PBS solution were placed in Eppendorf SafeLock™ tubes containing approximately 75 Ml 2.0mm zirconium oxide beads, 50 µL 1.0mm zirconium oxide beads, 50µL 0.5mm zirconium oxide beads with 6X sample volume lysis buffer consisting of 0.5M TEAB, 7M urea, 2M thiourea, 20% methanol and 4mM TCEP. The Bullet Blender Storm™ bead mill homogenizer (Next Advance Averill Park, NY) was then utilized to homogenize the sample at 4o Celsius at maximum agitation for 10 minutes. Thorough lysis and membrane disruption was achieved through use of a Barocycler® NEP2320 (Pressure Biosciences South Easton, MA) capable of applying a maximum hydrostatic pressure of 35,000 psi. The samples underwent thirty cycles at 36o Celsius 35,000 psi for 30 seconds and ~0 psi for 10 seconds. MMTS was added to a final concentration of 8mM.

Conclusions and Future Directions

1. Approximately 2,000 proteins were quantitatively detected in multiple iTRAQ runs characterizing COPD and lung cancer sample proteomes.
2. In a parsimonious approach we are able to profile the microbial proteome that is consistent with our previously published data. PubMed MID:23071781; PubMed Central PMCID:PMC3460539.

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Conclusions and Future Directions (continued)

3. The number of identifications from both proteogenomic and metaproteomic databases is limited yet consistent across multiple replicates.
4. Gene ontology demonstrates differentially expressed proteins represent aberrant cell movement, cell signaling and cell morphology.
5. Cav1 and PTRF are involved in caveolae formation and are highly dysregulated in patients with severe COPD and lung cancer.
6. Putative upstream regulators include ERBB2, ERG, MYCN, Tropomyosin and TGFβ1 in lung cancer with advanced COPD.

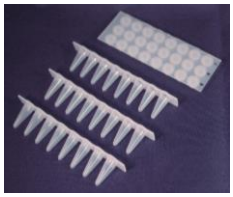
New Products from Pressure BioSciences for Enhanced Proteomic Sample Preparation

Pressure BioSciences, Inc., 14 Norfolk Ave., South Easton, MA 02375

Pressure BioSciences Inc. (PBI) focuses on development of products for biological sample preparation that utilize alternating ultra-high hydrostatic pressure – Pressure Cycling Technology (PCT). Pressure is a fundamental thermodynamic parameter that affects a wide range of molecular interactions. PBI's benchtop pressure cycling equipment functions by rapidly and repeatedly raising and lowering pressure in the reaction vessel from atmospheric to levels of up to 90,000 psi (690 MPa). Pressure cycling equipment and auxiliary tools such as the MicroPestles described here, have been shown to facilitate more quantitative extraction, more efficient digestion of hydrophobic membrane proteins, and more rapid enzymatic removal of N-linked glycans. Here we present an overview of two of PBI's newest products: The Barozyme HT48 for accelerated enzymatic digestion at 20,000 psi and PCT-HD for enhanced homogenization and digestion of small tissue samples at up to 45,000 psi.

Barozyme HT48

The new **Barozyme HT48** from Pressure BioSciences is a high throughput benchtop Pressure Cycling Technology (PCT) instrument designed for accelerated enzymatic digestion of proteins for mass spectrometry applications. The HT48 brings pressure-enhanced digestion to the industry standard microtiter plate format. The unit can process up to 48 samples at a time in convenient BaroFlex 8-well strips. Samples are exposed to cycles of high pressure at up to 20,000 psi (~1.4 Kbar or ~140 MPa) to accelerate digestion and improve detection of difficult to digest proteins.



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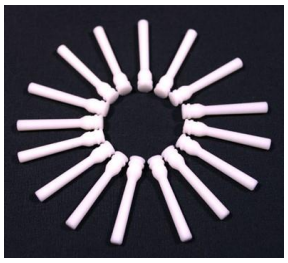
Click on Pictures for More Information

New Products from Pressure BioSciences for Enhanced Proteomic Sample Preparation: **Continued from Page 3, Column 1**

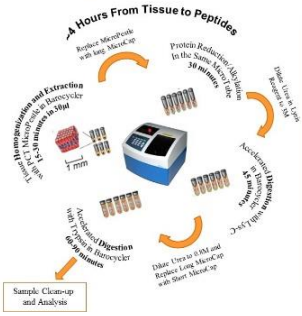
Barocycler NEP2320-Enhanced

To get the full power of PCT for protein extraction and accelerated digestion, PBI has developed the new Barocycler NEP2320-Enhanced. This new model can reach 45,000 psi, holds up to 16 MicroTubes at a time, and has a wider temperature range than the original NEP2320 model.



PCT-HD: A PCT-Enhanced Tissue Homogenization, Protein Extraction, and Digestion System for Mass Spectrometry Sample Preparation

PCT MicroPestles are designed for hands-off homogenization of minute solid tissue samples in 30µL of extraction reagent. Pressure cycling disrupts and homogenizes the tissue sample between the bottom of the MicroTube and the PCT MicroPestle tip, where it is repeatedly crushed on each pressure cycle.



Protein digests can be performed in the NEP2320-Enhanced in a fraction of the time required at ambient pressure. Trypsin digests typically require 1-2 hours, and often less, at 20,000 psi. Lys-C digests can be carried out at pressures up to 45,000 psi, often in less than 1 hour.

Summary

The PCT-HD System combines the hands-off multi-sample throughput of the PCT MicroPestle, with pressure-accelerated digestion, to significantly reduce processing time for MS workflows. Protein extraction with the PCT MicroPestle from biopsy-size tissues is rapid and convenient, providing excellent reproducibility and increased throughput. Pressure-accelerated digestion can be performed in the same MicroTube sample container to minimize sample transfers, improve yields, and save time. In addition, samples extracted and digested at high pressure may yield more peptides from difficult-to-extract and/or difficult-to-digest targets which may be underrepresented in traditional sample preparations

References

Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps

Tiannan Guo, Petri Kouvonen, Ching Chiek Koh, Ludovic C Gillet, Witold E Wolski, Hannes L Röst, George Rosenberger, Ben C Collins, Lorenz C Blum, Silke Gillessen, Markus Joerger, Wolfram Jochum & Ruedi Aebersold. Nature Medicine (2015) doi:10.1038/nm.3807

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