Journal of Proteomics & Bioinformatics
ISSN: 0974-276X
Kukreja et al., J Proteomics Bioinform 2012, S6
DOI: 10.4172/jpb.S6-001

Research Article Open Access

Immunosignaturing Microarrays Distinguish Antibody Profiles of Related Pancreatic Diseases

Muskan Kukreja, Stephen Albert Johnston and Phillip Stafford*

Center for Innovations in Medicine, Biodesign Institute, Arizona State University, Tempe, USA

Abstract

Immunosignaturing is a technology that allows the humoral immune response to be observed through the binding of antibodies to random sequence peptides. Profiles of the antibody repertoire produced during infection or during long-term chronic disease have proven to be informative for disease classification. An important unanswered question relative to this technology is whether different diseases that target the same organ and result in similar early phenotypes have similar or distinguishable immunosignatures. This question is of clinical relevance when considering patients who present with similar symptoms early during their disease. The pancreas is one such organ; diseases that affects this organ can cause the patient both broad and acute distress, with little to distinguish the disease source. If the cause were made clear without biopsy, and could be accomplished during routine monitoring, earlier intervention could improve health. Pancreatic cancer, chronic or acute pancreatitis, diabetes mellitus, hepatitis B or C infection, and other diseases can deeply affect the function of the pancreas, complicating diagnosis. We tested the immunosignaturing platform for its ability to resolve four different diseases that target the same organ; pancreatic cancer, pre-pancreatic cancer (panIN), type II diabetes and acute pancreatitis. These diseases were separated with >90% specificity from controls and from each other. We also describe a mathematical method that allows identification of 3 distinct components of an immunosignature: disease specific, 'housekeeping' and patient specific variation. The first component is useful in diagnosing disease, the second for baseline for the technology and third for monitoring changes in a healthy individual over time.

Keywords: Immunosignature; Immune profile; Random peptide microarray; Microarray proteomics; Pancreas disease; Pancreatic cancer; Type II diabetes; PanIN; Pancreatitis

Introduction

In theory, a given biomarker molecule can serve as a proxy for detecting and diagnosing disease and could be the most effective means of measuring drug efficacy and improving patient health [1]. One of the more ubiquitous technologies used for biomarker identification is mass spectrometry [2-4]. It has been widely used to search for diagnostics biomarkers, and the high sensitivity has made it useful for identifying informative biomarker molecules that associate with disease. This process of reducing biomarkers down to a single or few best candidates occasionally leads to overtraining, where highly precise biomarkers that work well in small cohorts become harder to correlate with large and diverse test populations [5]. It is becoming increasingly apparent that utilizing higher numbers of biomarkers simultaneously can relieve some of this 'low-feature-number' classification problem. Unfortunately, some attempts at using mass spectrometry to identify disease-associated mass spectrogram signatures have lead to skepticism about this concept [6,7].

One of the major drawbacks of serum-based biomarkers is dilution. The ability to detect small concentrations of protein or other biological compounds reproducibly has been tested and numerous issues with reproducibility and sensitivity have arisen [8-10]. Were there a candidate biomarker that was abundant, unaffected by age, sex, race, or genetic factors, different between healthy and sick persons and physically stable, the problem would become simpler. One such candidate is immunoglobulin molecules. Antibodies are amplified during an illness so dilution is less of a problem, they are differentially abundant between healthy and ill person, they are stable and are relatively unaffected by genetic factors. The humoral immune response can distinguish non-self antigens, modified self-antigens in the case of autoimmune disease, and neo-antigens in the case of many cancers [11-20].

In order to visualize changes in the antibody repertoire *en masse*, we developed a system we call 'immunosignaturing' [21-25]. We capture and display the complexities of humoral immunity using a microarray of random-sequence peptides. The system works for any isotype and has detected autoimmune disease, cancer, infectious disease, and chronic disease. The microarray is commercially printed to reduce variability and cost; technical reproducibility between replicate arrays averages 0.95 but is often >0.99.

While we have seen clear distinctions between disease and healthy controls, we had not tested the idea that immunosignatures might be quite similar if a general inflammation response is raised for a particular target organ, though the primary disease might be quite different. We tested four different diseases that each affects the pancreas, leading to similar acute symptoms, but leading to substantially different late-stage symptoms [26-28]. Clinically, this would aid patients who present with similar early symptoms. If the immunosignatures revealed distinctions regardless of the common symptoms, it would enhance early intervention and could improve patient health. Is a general inflammation response driving the early humoral immune response in pancreatic disease or are antibody profiles distinct enough to predict disease? We examined patients with pancreatic cancer, pancreatitis, a pre-pancreatic cancer condition known as panIN, and type II diabetes.

***Corresponding author:** Dr. Phillip Stafford, Center for Innovations in Medicine, Biodesign Institute, Arizona State University, Tempe, USA, Tel: 480 7270 795; E-mail: phillip.stafford@asu.edu

Received November 10, 2011; **Accepted** December 05, 2011; **Published** January 30, 2012

Citation: Kukreja M, Johnston SA, Stafford P (2012) Immunosignaturing Microarrays Distinguish Antibody Profiles of Related Pancreatic Diseases. J Proteomics Bioinform S6:001. doi:10.4172/jpb.S6-001

Copyright: © 2012 Kukreja M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Pancreatic cancer refers to a malignant neoplasm of the pancreas. About 95% of pancreatic tumors arise within the exocrine component of pancreas [29,30]. Pancreatitis is inflammation of the pancreas due to ectopic or restricted activation of enzymes [31]. PanIN stands for Pancreatic Intraepithelial Neoplasia and is the initial stage of pancreatic cancer [32], also considered a non-carcinomic dysplasia. Type II diabetes is a chronic condition in which body has insulin resistance and deficiency resulting in high glucose level in the body [33]. There has been no complete survey of pancreatic diseases in the context of humoral immunity, but there is increasing evidence that patients with one pancreas disease have higher risk of a subsequent pancreas disease due to shared pathology and immunological involvement including autoimmunity [34-39].

An immunosignature is the cumulative information from selected random-sequence peptides that bind differentially to antibodies from healthy controls vs. disease patients. Peptides are selected using statistical measures (t-test or ANOVA). Each signature, whether at a single time point from multiple patients with the same disease or from a single patient across multiple time points, can be considered a vector. This vector has three major components: 1) the disease component, 2) the unchanged component and 3) the personal variation component.

The first component consists of peptides that show a relative 'up' or 'down' response during the course of disease compared to healthy controls. A simple t-test with multiple testing corrections applied can identify peptides that are reproducibly higher or lower in patients vs. controls. Typically, biomarkers are missing in healthy controls and begin to appear in patients with a given disease. In immunosignaturing, signals can be either higher *or* lower between disease and control; this is not typical for the biomarker paradigm.

The second component represents peptides that do not change between disease and healthy individuals. These antibodies may are not activated during disease, and may simply be circulating or basal level antibodies produced against a common infection or vaccination. This component helps quantify the part of the immunosignature that does not vary during the course of disease, helping to establish a baseline of variance and dynamic range.

The third component is personal variation and signifies the behavior of an individual's own immune system. This component is necessary when establishing a baseline for a patient over time. These three components are extracted mathematically from a given immunosignature. We present these three components in the context of our analysis of four pancreas diseases.

Materials and Methods

Microarray

The CIM 10K array is a 2-up microarray containing 10,000 random-sequence 20-mer peptides attached via a maleimide reaction to the NH_3 terminal sulfur of cysteine, creating a covalent attachment [21-25]. The CIM 10K microarray is available to the public at www.peptidemicroarraycore.com.

Sample processing

Plasma samples from patients and healthy controls were stored at -80°C until needed. Samples were aliquoted and refrozen at -20°C. Samples were diluted at 1:500 in sample buffer (1xPBS, 0.5% Tween20, 0.5% Bovine Serum Albumin (Sigma, St. Louis, MO)) and exposed to the array according to the protocol in [24]. Antibodies were detected with 5nm Alexafluor 647-labeled streptavidin (Invitrogen, Carlsbad, CA), which bound 5nM biotinylated anti-human secondary antibody (Novus anti-human IgG (H+ L), Littleton, CO). Microarrays were scanned and converted to tabular data as in [24]. Median foreground signal was used as the value which best-represented binding of antibody to peptide.

Samples

Center for Innovations in Medicine, Biodesign Institute, Arizona State University has an existing IRB 0912004625, which allows unfettered analysis of blinded samples from collaborators.

Type II diabetes: 17 plasma samples which had poorly controlled type II diabetes with no history of CHF (Congestive Heart Failure) and MF (Myocardial Infraction).

Pancreatic cancer: This set contains 13 plasma samples from patients with ductal adenocarcinoma of the pancreas.

Pancreatitis: This set contains 10 plasma samples of patients with refractory pancreatitis.

PanIN: This set contains 5 plasma samples. Samples were obtained from a single family with history of pancreatic cancer. Samples were diagnosed with a pre-stage of pancreatic cancer.

Common Controls: This set contain 16 plasma samples from the diabetes study.

Data analysis: The raw tabular data were imported to GeneSpring 7.3.1 (Agilent, Santa Clara, CA). Data were median normalized per array and $\log_{10}$ transformed. Feature selection used t-test with family-wise Multiple Error correction of 5% (FWER=5%). For multiple groups we used 1-way fixed-effects ANOVA, FWER=5%. *All p-values presented are after FWER correction*. The three components were selected as follows: component 1 (disease component) was selected by using t-test. Component 2 (unchanged component) was selected by ANOVA (FWER = 5%) on all samples including controls and disease, these peptides are the ones which were not selected by ANOVA signifying no significant change over samples excluding those peptides that were selected for component 1. Component 3 (personal variation) are those peptides that passed ANOVA (FWER= 5%) on all samples including disease and controls.

Data classification: For classification, Naïve Bayes and leave one out cross-validation was used. Classification was performed in open source JAVA software WEKA [40].

Results

Analysis of three (disease component, housekeeping and personal variation) immunosignaturing component: 10 samples of pancreatitis (PC), 5 samples of panIN (PN), 17 samples of type II diabetes (T2D), 13 samples of pancreatic cancer (PC) and 16 samples of healthy controls were run in duplicate on the 10K peptide microarrays. Technical replicates with Pearson's correlation coefficient <0.90 were discarded. For each disease, the three components listed in Table 1 were determined as a number of peptides at a given p-value.

Features that comprise each of the three immunosignaturing components were identified at an adjusted p<0.05 and are presented in Table 1. The disease components of pancreatic cancer and panIN contribute from 10-20% (lowest to highest) to the net immunosignaturing vector, while the disease component of type II diabetes and pancreatitis contributes little (< 3%) to the net vector. The unchanged components within type II diabetes, pancreatitis and

Citation: Kukreja M, Johnston SA, Stafford P (2012) Immunosignaturing Microarrays Distinguish Antibody Profiles of Related Pancreatic Diseases. J Proteomics Bioinform S6:001. doi:10.4172/jpb.S6-001

panIN contribute 17-23% (average=19%) to the net vector while that of pancreatic cancer contributes <5%. The personal variation component comprises most of the immunosignaturing net vector in pancreatic cancer, with 60-80% (average=76%).

Classification performance: Each of the diseases tested were subjected to a test/training analysis consisting of feature selection (component 1) followed by classification using Naïve Bayes and leave-one-out cross-validation. Naïve Bayes treats features as completely independent sources of information, which has advantages for a system like immunosignaturing, less so for expression or SNP microarrays where there is a biological connection across features. The performance from the peptides that compose the disease immunosignaturing component is shown in Table 2, where accuracy was on average 93%.

Immunosignaturing of pancreas related diseases

We established the primary features that distinguish disease vs. control, presented in Table 2, along with the p-value cutoff, classification accuracy, specificity and sensitivity using Naïve Bayes error and leave one out cross validation. We then asked of the peptides that are changed between control and each disease, how many are up or down compared to controls. Of note, detecting informative signals less than normal is a feature not possible for ELISA-type assays. For type II diabetes, most (~90%) of the peptides were up compared to controls while for panIN, the same percentage were down. For pancreatic cancer and pancreatitis, between 50 and 60 % were down compared to controls while 40 to 50% of the selected peptides were up. Given the initial question of similarity between diseases that affect the same target organ, and how much of an immunosignature is derived from a general inflammation response, we asked how many of these peptides were in common, and how well could we distinguish the diseases from each other.

# peptides/10,000	Type 2 diabetes	Pancreatic cancer	Pancreatitis	PanIN
Disease component	92	1058	258	1696
Unchanged component	1700	536	2235	2041
Personal variation	8208	8406	7507	6263

Table 1: Distribution of three components for pancreas disease. Breakdown of peptides specific to the three components at p<0.05 with FWER=5%.

	T2D Vs Controls	PC Vs. Controls	PT Vs. Controls	PN Vs. Controls
# peptides	92 (p < 0.05)	244 (p <0.005)	258 (p <0.05)	233 (p<0.0005)
Accuracy	87.88 %	93.10 %	96.15 %	95.24 %
Specificity	93.75 %	93.8 %	100 %	100 %
Sensitivity	82.4 %	92.3 %	90 %	80 %

Table 2: Classification performance for predicting each disease with peptides from disease component. Table showing classification accuracy, specificity and sensitivity for each pancreas disease versus common controls. T2D = type II diabetes, PC = pancreatic cancer, PT = pancreatitis, PN = panIN.

	T2D &PC	T2D & PT	T2D & PN	PC & PT	PC & PN	PT & PN
No of peptides	248 (p<0.05)	134 (p<0.05)	163 (p<0.0005)	244 (p<0.05)	42 (p<0.05)	150 (p<0.005)
Accuracy	90 %	92.59 %	95.45 %	95.65 %	94.44 %	93.33 %

Table 3: Classification accuracy between each pancreas disease. Classification accuracy between each disease without using healthy controls. T2D = type II diabetes, PC = pancreatic cancer, PT = pancreatitis, PN = panIN.

Features that distinguish each disease from every other were identified using corrected t-test and presented in Table 3. The accuracy of Naïve Bayes classification using leave-one-out cross validation is shown for each comparison is shown along with the p-value cutoff for each comparison.

Notably at p<0.05, the differences between type 2 diabetes, pancreatic cancer and pancreatitis are small (< 2% of the peptides are distinct) while the difference between pancreatic cancer and panIN is <1%. Between type 2 diabetes and panIN the differences were greater, at 15% of the peptides. Between pancreatitis and panIN, 11% but we were able to achieve > 90% classification accuracy for each disease comparison. We then asked exactly how similar the peptides were that distinguished each disease.

Similarities among 'disease component' of pancreas disease

The top 200 features that distinguished each disease from controls were identified, regardless of the p-value cutoff (top 200 peptides from disease component). Combinatorially, the probability of seeing *r* or more peptides common among 200 from each disease component by chance is obtained from equation 1, where *r* is the set of peptides selected to distinguish any given comparison, p is no. of peptides selected out of total (200) and n is total no of peptides (10,000). For *r* > 10, this probability is <1%. Equation 1 states this probability.

$$Probability = 1 - \left(\frac{\sum_{i=0}^{r=1} \binom{p}{i} * \binom{n-p}{p-i}}{\binom{p}{i}} \right)$$

We examined these 200 peptides for degree of similarity; we selected the same number of peptides for each disease comparison so that the overlap will be proportional. Figure 1A shows the overlap between each disease component. The overlap of type 2 diabetes with pancreatic cancer is high significant (22 peptides); the common peptides are up compared to controls. The overlap of type II diabetes with pancreatitis and panIN is small (<10 peptides). The overlap of pancreatic cancer with pancreatitis and panIN is high (~50 peptides) and these common peptides are down compared to controls. Figure 1B shows the overlap among pancreatic cancer, pancreatitis and panIN. This overlap is high (17 peptides) and all common peptides are down regulated compared to controls.

Towards a general diagnostic of pancreas-related diseases

We asked whether we could construct a small diagnostic array that was inexpensive, reproducible, and would allow multiple tests per microarray. To do this we need far fewer than 10,000 peptides. Features that distinguish each of the four different diseases from controls were identified at an adjusted p<0.025. Table 4 shows the classification accuracy of 643 peptides using Naïve Bayes and leave one out cross validation. This set includes peptides that are informative for pancreas disease we examined, but those peptides may be non-informative for another disease comparison. This is a major hurdle when multiplexing immunological tests on the same device. However, we achieved >90% accuracy, specificity and sensitivity using peptides that will fit 24 times on a standard microarray slide enabling higher throughput and far inexpensive assay costs.

Discussion

We used immunosignature technology to examine different diseases that target the same organ, the pancreas. We tested whether

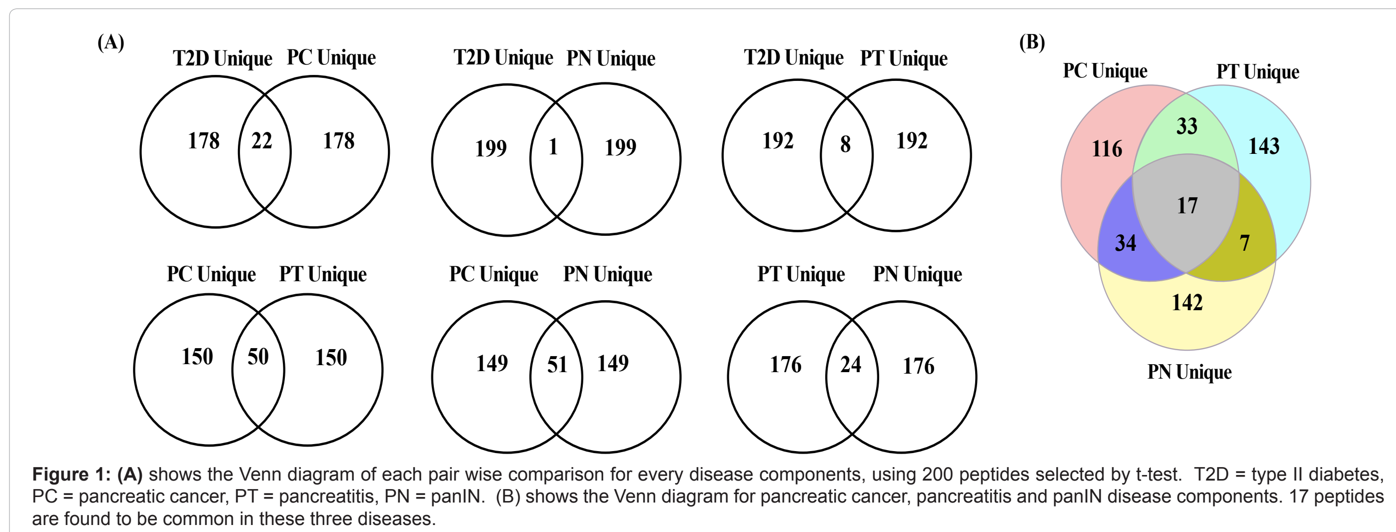

Figure 1: (A) shows the Venn diagram of each pair wise comparison for every disease components, using 200 peptides selected by t-test. T2D = type II diabetes, PC = pancreatic cancer, PT = pancreatitis, PN = panIN. (B) shows the Venn diagram for pancreatic cancer, pancreatitis and panIN disease components. 17 peptides are found to be common in these three diseases.

there was a general immunological affect that might render signatures from patients with pancreas disease very similar by looking for overlapping peptides. We found a distinct set of peptides that could classify each of the 4 diseases, but there were also peptides that were common across diseases. We found that there were different numbers of peptides that were in common across diseases and different numbers of peptides that were uniquely personal to each patient and different numbers of peptides that were unchanged within the disease class.

Initially we investigated whether each disease was distinct compared to healthy controls. We obtained >95% specificity on average (93.75, 93.8, 100, 100 for type II diabetes, pancreatitis, panIN and pancreatic cancer respectively). Next we tested whether each disease was distinct from each other, and in this case we obtained >90% classification accuracy. We thus show that each disease, although affecting the pancreas to some extent, also has unique immunological characteristics.

We then looked for similarities between each pancreas disease. The disease component (the part of the signature that defines the uniqueness of each disease) was used to examine the commonality between type II diabetes and pancreatic cancer. These two diseases share a significant portion of this component, perhaps caused by common immunological stimuli. All the common peptides were up compared to controls, suggesting a common antigen. Similarly, there was significant similarity across pancreatic cancer, pancreatitis and panIN. All common peptides were down compared to controls suggesting that there may be some immune suppression in these diseases. The different pancreas diseases have their own unique signatures but also share portions of their 'disease component' (component 1). The third component (personal variation) was found to contribute differently across the diseases. This component is important when using immunosignaturing for monitoring health status over time. The patient uniqueness was established as those peptides which differ from person to person, excluding the disease specific (component 1) and housekeeping or 'normal component' (component 2) peptides. We found that a range between 60 and 85% of the total 10,000 different peptides were individual specific (component 3) when examining these 4 diseases.

	Pancreas disease Vs Common controls
No. of peptides	643 (p <0.025)
Accuracy	91.8 %
Specificity	93.8 %
Sensitivity	91.8 %

Table 4: Towards a general diagnostics for pancreas related diseases. Classification accuracy, specificity and sensitivity for pancreas disease versus controls.

Finally, in order to establish the potential of this technology for creating a diagnostic, we tested whether mixing peptides specific to a number of diseases would detrimentally affect the classification performance. We chose 643 peptides that differentiate these 4 diseases. These peptides could be printed on a 24-up microarray, which allows much cheaper per-assay cost and far higher throughput. Mixing the most informative peptides for distinguishing each disease from controls and from each other yielded >90% classification accuracy. The fact that a pattern of peptides can be found that reliably distinguishes a disease from unrelated individuals is remarkable, but the presence of at least 3 distinguishable components within that signature lends credence to the fact that antibodies are highly tuned to the health status of an individual.

Acknowledgments

We are thankful to Teri Brentnall, University of Washington, Seattle, for providing samples of pancreatic cancer, pancreatitis, panIN and also to Molecular Biosignatures Analysis Unit at Biodesign Institute, ASU for providing type II diabetes samples and common controls. This work was supported by discretionary funds from SAJ.

References

1. Weston AD, Hood L (2004) Systems Biology, Proteomics, and the Future of Health Care:‚Äâ Toward Predictive, Preventative, and Personalized Medicine. J Proteome Res 3: 179-196.

2. Ackermann B L, Hale JE, Duffin KL (2006) The Role of Mass Spectrometry in Biomarker Discovery and Measurement. Curr Drug Metab 7: 525-539.

3. Lamont RJ, Meila M, Xia Q, Hackett M (2006) Mass spectrometry-based proteomics and its application to studies of Porphyromonas gingivalis invasion and pathogenicity. Infect Disord Drug Targets 6: 311-325.

4. Li J, Orlandi R, White CN, Rosenzweig J, Zhao J, et al. (2005) Independent Validation of Candidate Breast Cancer Serum Biomarkers Identified by Mass Spectrometry. Clin Chem 51: 2229-2235.

5. Kiehntopf M, Siegmund R, Deufel T (2007) Use of SELDI-TOF mass spectrometry for identification of new biomarkers: potential and limitations. Clin Chem Lab Med 45: 1435-1449.

6. Chapman K (2002) The ProteinChip Biomarker System from Ciphergen Biosystems: a novel proteomics platform for rapid biomarker discovery and validation. Biochem Soc Trans 30: 82-87.
7. Davies HA (2000) The ProteinChip System from Ciphergen: a new technique for rapid, micro-scale protein biology. J Mol Med (Berl) 78: B29.
8. Barbosa F Jr, Tanus-Santos JE, Gerlach RF, Parsons PJ (2005) A critical review of biomarkers used for monitoring human exposure to lead: advantages, limitations, and future needs. Environ Health Perspect 113: 1669-1674.
9. Diamandis EP, van der Merwe DE (2005) Plasma protein profiling by mass spectrometry for cancer diagnosis: opportunities and limitations. Clin Cancer Res 11: 963-965.
10. Elias JE, Haas W, Faherty BK, Gygi SP (2005) Comparative evaluation of mass spectrometry platforms used in large-scale proteomics investigations. Nat Methods 2: 667-675.
11. Ada GL, Jones PD (1986) The immune response to influenza infection. Curr Top Microbiol Immunol 128: 1-54.
12. Brichory F, Beer D, Le Naour F, Giordano T, Hanash S (2001) Proteomics-based identification of protein gene product 9.5 as a tumor antigen that induces a humoral immune response in lung cancer. Cancer Res 61: 7908-7912.
13. Brydak LB, Machala M (2000) Humoral Immune Response to Influenza Vaccination in Patients from High Risk Groups. Drugs 60: 35-53.
14. Cox RJ, Brokstad KA, Zuckerman MA, Wood JM, Haaheim LR, et al. (1994) An early humoral immune response in peripheral blood following parenteral inactivated influenza vaccination. Vaccine 12: 993-999.
15. DiFronzo LA, Gupta RK, Essner R, Foshag LJ, O'Day SJ, et al. (2002) Enhanced humoral immune response correlates with improved disease-free and overall survival in American Joint Committee on Cancer stage II melanoma patients receiving adjuvant polyvalent vaccine. J Clin Oncol 20: 3242-3248.
16. Hooks JJ, Moutsopoulos HM, Geis SA, Stahl NI, Decker JL, et al. (1979) Immune Interferon in the Circulation of Patients with Autoimmune Disease. N Engl J Med 301: 5-8.
17. Lennon VA, Lindstrom JM, Seybold ME (1976) Experimental autoimmune myasthenia gravis: cellular and humoral immune responses. Ann N Y Acad Sci 274: 283-299.
18. Sreekumar A, Laxman B, Rhodes DR, Bhagavathula S, Harwood J, et al. (2004) Humoral immune response to alpha-methylacyl-CoA racemase and prostate cancer. J Natl Cancer Inst 96: 834-843.
19. Stockert E, Jager E, Chen YT, Scanlan MJ, Gout I, et al. (1998) A survey of the humoral immune response of cancer patients to a panel of human tumor antigens. J Exp Med 187: 1349-1354.
20. Wilder RL (1995) Neuroendocrine-immune system interactions and autoimmunity. Annu Rev Immunol 13: 307-338.
21. Boltz KW, Gonzalez-Moa MJ, Stafford P, Johnston SA, Svarovsky SA (2009) Peptide microarrays for carbohydrate recognition. Analyst 134: 650-652.
22. Brown J, Stafford P, Johnston S, Dinu V (2011) Statistical Methods for Analyzing Immunosignatures. BMC Bioinformatics 12: 349.
23. Halperin RF, Stafford P, Johnston SA (2011) Exploring antibody recognition of sequence space through random-sequence peptide microarrays. Mol Cell Proteomics 10: M110.000786.
24. Legutki JB, Magee DM, Stafford P, Johnston SA (2010) A general method for characterization of humoral immunity induced by a vaccine or infection. Vaccine 28: 4529-4537.
25. Restrepo L, Stafford P, Magee DM, Johnston SA (2011) Application of immunosignatures to the assessment of Alzheimer's disease. Ann Neurol 70: 286-295.
26. Dugernier TL, Laterre PF, Wittebole X, Roeseler J, Latinne D, et al. (2003) Compartmentalization of the Inflammatory Response during Acute Pancreatitis: Correlation with Local and Systemic Complications. Am J Respir Crit Care Med 168: 148-157.
27. Fineberg SE, Kawabata T, Finco-Kent D, Liu C, Krasner A (2005) Antibody response to inhaled insulin in patients with type 1 or type 2 diabetes. An analysis of initial phase II and III inhaled insulin (Exubera) trials and a two-year extension trial. J Clin Endocrinol Metab 90: 3287-3294.
28. Orchekowski R, Hamelinck D, Li L, Gliwa E, VanBrocklin M, et al. (2005) Antibody Microarray Profiling Reveals Individual and Combined Serum Proteins Associated with Pancreatic Cancer. Cancer Res 65: 11193-11202.
29. Hruban RH, Iacobuzio-Donahue C, Wilentz RE, Goggins M, Kern SE (2001) Molecular pathology of pancreatic cancer. Cancer J 7: 251-258.
30. Li D, Xie K, Wolff R, Abbruzzese JL (2004) Pancreatic cancer. The Lancet 363: 1049-1057.
31. Saluja AK, Steer ML (1999) Pathophysiology of Pancreatitis. Digestion 60: 27-33.
32. Hruban RH, Goggins M, Parsons J, Kern SE (2000) Progression model for pancreatic cancer. Clin Cancer Res 6: 2969-2972.
33. Katsilambros N, Diakoumopoulou E, Ioannidis I, Liatis S, Makrilakis K, et al. (2006) Pathophysiology of Type 2 Diabetes, in Diabetes in Clinical Practice: John Wiley & Sons, Ltd. 43-58.
34. Ekbom A, McLaughlin JK, Karlsson BM, Nyren O, Gridley G, et al. (1994) Pancreatitis and Pancreatic Cancer: a Population-Based Study. J Natl Cancer Inst 86: 625-627.
35. Huxley R, Ansary-Moghaddam A, Berrington de Gonzalez A, Barzi F, Woodward M (2005) Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. Br J Cancer 92: 2076-2083.
36. Lowenfels AB, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, et al. (1993) Pancreatitis and the Risk of Pancreatic Cancer. N Engl J Med 328: 1433-1437.
37. Deshpande V, Mino-Kenudson M, Brugge W, Lauwers GY (2005) Autoimmune Pancreatitis: More Than Just a Pancreatic Disease?A Contemporary Review of Its Pathology. Arch Pathol Lab Med 129: 1148-1154.
38. Inoue H, Miyatani H, Sawada Y, Yoshida Y (2006) A Case Of Pancreas Cancer With Autoimmune Pancreatitis. Pancreas 33: 208-209.
39. Okazaki K, Chiba T (2002) Autoimmune related pancreatitis. Gut 51: 1-4.
40. Hall M, Frank E, Holmes G, Pfahringer B, Reutemann P, et al. (2009) The WEKA data mining software: an update. ACM SIGKDD Explor Newsl 11: 10-18.

This article was originally published in a special issue, **Microarray Proteomics** handled by Editor(s). Dr. Qiangwei Xia, University of Wisconsin-Madison, USA