HHS Public Access

Author manuscript

Circulation. Author manuscript; available in PMC 2019 March 20.

Published in final edited form as:

Circulation. 2018 March 20; 137(12): 1270–1277. doi:10.1161/CIRCULATIONAHA.117.029443.

Application of large scale aptamer-based proteomic profiling to "planned" myocardial infarctions

Jaison Jacob, PhD[1,*], **Debby Ngo, MD**[2,3,*], **Nancy Finkel, MS**[1], **Rebecca Pitts, MS**[1], **Scott Gleim**[1], **Mark D. Benson, MD, PhD**[4], **Michelle J. Keyes, PhD**[3], **Laurie A. Farrell, RN**[3], **Thomas Morgan, MD**[1], **Lori L. Jennings, PhD**[1,**], and **Robert E. Gerszten, MD**[2,5,**]

[1]Novartis Institute for BioMedical Research, Chemical Biology and Therapeutics, Cambridge MA

[2]Division of Pulmonary, Critical Care and Sleep Medicine, Beth Israel Deaconess Medical Center, Boston, MA

[3]Cardiovascular Research Center, Beth Israel Deaconess Medical Center, Boston, MA

[4]Cardiovascular Division, Brigham and Women's Hospital, Boston, MA

[5]Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, Boston, MA

Abstract

Background—Emerging proteomic technologies using novel affinity-based reagents allow for efficient multiplexing with high sample throughput. To identify early biomarkers of myocardial injury, we recently applied an aptamer-based proteomic profiling platform that measures 1,129 proteins to samples from patients undergoing septal alcohol ablation for hypertrophic cardiomyopathy, a human model of "planned myocardial injury" (PMI). Here we examined the scalability of this approach using a markedly expanded platform to study a far broader range of human proteins in the context of myocardial injury.

Methods—We applied a highly multiplexed, expanded proteomic technique that uses single stranded DNA aptamers to assay 4,783 human proteins (4,137 distinct human gene targets) to derivation and validation cohorts of PMI, to individuals with spontaneous myocardial infarction (SMI), and to at-risk controls.

Results—We found 376 target proteins that significantly changed in the blood after PMI in a derivation cohort (n=20; $P < 1.05E-05$, one-way repeated measures ANOVA, Bonferroni-threshold). Two hundred forty-seven of these proteins were validated in an independent PMI cohort (n=15; $P < 1.33E-04$, one-way repeated measures ANOVA); > 90% were directionally consistent and reached nominal significance in the validation cohort. Among the validated proteins that were increased within 1 hour after PMI, 29 were also elevated in patients with spontaneous myocardial infarction (n=63; $P < 6.17E-04$). Many of the novel markers identified in our study are

Correspondence: Robert E. Gerszten, MD, Division of Cardiovascular Medicine, Beth Israel Deaconess Medical Center, 330 Brookline Ave, Boston, MA 02115, Phone: 617-632-7647, Fax: 617-643-3461, rgerszte@bidmc.harvard.edu or Lori L. Jennings, PhD, Chemical Biology and Therapeutics, Novartis Institute for BioMedical Research, 22 Windsor St, Cambridge, MA 02139, Phone: 617-871-5625, lori.jennings@novartis.com.

*denotes shared first authorship;

**enotes shared senior authorship

DISCLOSURES: JJ, SG, NF, RP, TM and LLJ are employees of Novartis and some are also stockholders of Novartis.

intracellular proteins not previously identified in the peripheral circulation or have functional roles relevant to myocardial injury. For example, the cardiac LIM protein cysteine and glycine-rich protein 3 (CSRP3) is thought to mediate cardiac mechanotransduction and stress responses while the mitochondrial ATP synthase F0 subunit component (ATP5J) is a vasoactive peptide upon its release from cells. Finally, we performed aptamer-affinity enrichment coupled with mass spectrometry to technically verify aptamer specify for a subset of the new biomarkers.

Conclusion—Our results demonstrate the feasibility of large scale aptamer multiplexing at a level that has not previously been reported and with sample throughput that greatly exceeds other existing proteomic methods. The expanded aptamer-based proteomic platform provides a unique opportunity for biomarker and pathway discovery following myocardial injury.

Keywords

proteins; cardiovascular disease; myocardial infarction

INTRODUCTION

Obstacles for comprehensive blood proteomic profiling include the immense size and structural heterogeneity of circulating proteins, as well as the broad range of abundance levels.[1–3] Applications of mass spectrometry based profiling to plasma are limited by complex analytical steps that severely restrict sample throughput.[4] By contrast, multiplexing of targeted methods based on capture and detection of specific proteins may afford advantages for large scale efforts to characterize human samples. However, many antibody-based assays are limited by cross-reactivity which precludes large scale multiplexing.[5]

To address this limitation, investigators have turned to alternative affinity-based reagents, including DNA aptamers. Whether aptamer reagents are well-suited for efficient multiplexing of thousands of proteins at high sample throughput remains to be tested. As a proof of principle, we recently applied a proteomic profiling platform with multiplexed aptamers[6] to samples from patients undergoing septal alcohol ablation for hypertrophic cardiomyopathy.[6] This human model of “planned” myocardial injury (PMI), in which each individual serves as their own biologic control, reproduces key clinical features of “spontaneous” MI, including chest pain, electrocardiographic changes and wall motion abnormalities, as well as the release of established markers of myocardial injury.[7, 8] Upon screening 1,129 proteins on the original platform, we validated 79 proteins that changed in the context of myocardial injury. Here we tested the scalability of this approach with a markedly expanded platform containing ~ 5,000 aptamers targeting a far broader range of analytes. The expanded platform measures many intracellular proteins not previously assayed in the blood, as well as many low-abundance secreted proteins not previously targeted by aptamer-based reagents. Thus, the substantially expanded platform provides an unprecedented opportunity for novel discovery of proteins that are altered following myocardial injury.

METHODS

A complete list of all proteins that change significantly after "planned MI" are included in Supplemental Table 1; a complete list of proteins that differ between spontaneous MI patients and controls are included in Table 1 and Supplemental Table 2. The analytic methods will be made available to other researchers for purposes of reproducing the results or replicating the procedure. However, a subset of the study materials (e.g., DNA aptamers) is proprietary to Novartis and Somalogic and investigators are encouraged to contact the corresponding PIs for questions.

Human Studies Participants

A total of thirty-five patients undergoing PMI using alcohol septal ablation for the treatment of symptomatic hypertrophic cardiomyopathy were included in this study (20 in the derivation cohort; 15 in a distinct validation cohort). Inclusion criteria for this cohort and details of the procedure have been previously described.[9–11] Blood was drawn at baseline, 10 minutes, 1 hour and 24 hours after injury. To determine the effects of the catheterization procedure alone, including heparin administration, we studied ten individuals who underwent cardiac catheterization without myocardial injury as controls (eight patients undergoing cardiac catheterization for patent foramen ovale closure and two patients undergoing cardiac catheterization for PMI who did not undergo septal ablation for anatomical reasons).

We also enrolled 20 patients undergoing emergent cardiac catheterization for acute ST-segment elevation spontaneous MI within 8 hours of symptom onset. Femoral venous blood samples were obtained in the coronary catheterization suite upon initial presentation. Peripheral blood samples from 43 at-risk patients with negative standard Bruce protocol exercise tests were used as controls for spontaneous MI.[12]

All blood samples were collected in K2EDTA-treated tubes, centrifuged within 15 minutes at 2000*g* for 10 min to pellet cellular elements and subsequently stored at −80 °C.

Human study protocols were approved by the Institutional Review Boards of Beth Israel Deaconess Medical Center and Massachusetts General Hospital. Informed consent was obtained from all participants.

Proteomic assay overview

A proteomic profiling platform has been developed that applies 5,034 single-stranded DNA aptamers (validated SOMAmers™) to assay 4,783 human proteins (4,137 distinct human gene targets), 48 non-human vertebrate proteins and 203 bacterial and viral protein targets covering a broad range of protein functional classifications (Supplemental Figure 1). The intra-assay and inter-assay CVs are presented in the **Results** section. As compared to the prior iteration of the platform, this version covers a broader array of intracellular proteins (approximately 30%).

Statistical analyses

For the PMI studies, all protein values were log transformed due to nonnormal distributions as determined by the Kolomogorov-Smirnov and Shapiro-Wilk normality tests. For the PMI and cardiac catheterization control studies, one-way repeated measures ANOVA was used to test differences in protein levels across time points (baseline, 10 minutes, 1 hour, and 24 hours). All reported p-values are global p-values, i.e., indicating whether protein levels change significantly between baseline and any of the time points. The multivariate adjustment was used if sphericity was violated. In the derivation cohort, we used a Bonferroni-corrected P threshold < 1.05E-05 (0.05/4783 proteins unaffected in control catheterization, see below) and repeat Bonferroni-corrected P threshold < 1.33E-04 (0.05/376) aptamers in the PMI validation cohort. For the spontaneous myocardial infarction (SMI) case-control analysis, a Wilcoxon rank-sum test was used with a Bonferroni-corrected P threshold < 6.17E-04 (0.05/81 validated proteins shown to increase within 1 hour after myocardial injury in the PMI cohort). All analyses were performed with SAS Software version 9.3 (SAS Institute, Cary, NC).

RESULTS

Protein changes in peripheral plasma of PMI patients

To first assess reproducibility of the platform, we embedded pooled plasma control samples within and across experimental plates to document intra-and inter-experimental coefficients of variance (CVs). The intra-assay and inter-assay CVs (applying performance characteristics for 95% of the probe content in plasma) were 7.45 and 6.49, respectively. Inter-assay CVs were based on plates that were run up to 21 days apart. To assess biological variability, we compared two plasma samples from normal controls collected 10 minutes apart (n=12), which yielded a median intraclass correlation of 0.86 across the entire analytes assayed.

Clinical characteristics of the PMI study patients are detailed in Supplemental Table 3. Using each individual as their own biologic control, we performed proteomic profiling at baseline, 10 minutes, 1 hour and 24 hours after injury. We identified a total of 376 proteins that were significantly changed within 24 hours post injury in a derivation cohort of 20 patients (Bonferroni-adjusted P < 1.05E-05, one-way repeated measures ANOVA). Of note, we identified 2,027 aptamer-protein pairs that were influenced by the catheterization procedure alone with similar directional changes in PMI, likely due to heparin treatment (*nominal* P < 0.05). These proteins were therefore excluded from the analyses due to possible confounding effects. In a validation cohort of 15 patients, changes in 247 proteins exceeded a repeat Bonferroni threshold (P < 1.33E-04, one-way repeated measures ANOVA). Ninety percent of the proteins changes seen in the derivation cohort were directionally consistent and reach at least nominal significance (P < 0.05) in the smaller validation cohort. Table 2 details a subset of the proteins found to be significant in both derivation and validation cohorts that increased by greater than 30% within 10 minutes after PMI in both cohorts. All validated proteins are listed in Supplemental Table 1. See Supplemental Table 4 for clinical characteristics of the catheterization controls.

The kinetic changes of several representative proteins are illustrated in Figure 1. Using the expanded platform, we confirmed increases in well-established clinical markers of myocardial injury including troponin I and CK-MB, as well as other biomarkers previously identified by our group and others such as fatty acid binding protein (FABP)[13, 14] and malate dehydrogenase 1 (MDH1).[6] Many of these protein changes were novel in the context of early myocardial injury (see Table 2 and Supplemental Table 1), including a mitochondrial ATP synthase F0 subunit component (ATP5J),[15] mitochondrial 2–4-dienoyl-CoA reductase 1 that is important in fatty acid beta oxidation (DECR1),[16] and a muscle-specific adenylosuccinate synthase that plays a role in purine nucleotide metabolism (ADSSL1).[17]

Validation of candidate protein markers in spontaneous myocardial infarction

To better establish the clinical relevance of the observed changes, we profiled a cohort of spontaneous myocardial infarction (SMI) patients (n=20), as well as at-risk individuals without ischemia as determined by exercise stress testing (n=43, clinical characteristics in Supplemental Table 4). Because the timing of sample collection relative to SMI onset was variable (6.0 ± 1.9 h), we focused on the PMI-derived candidate protein markers that were elevated within one hour after injury, the time point in the PMI series closest to the time of SMI presentation. Of the 81 proteins that were significant and elevated within 1 hour after PMI in both the derivation and validation cohorts, 29 were also elevated in SMI (repeat Bonferroni adjusted $P < 6.17E-04$; WRS, Table 1). Among the many novel findings that were concordantly increased in both PMI and SMI were ADSSL1, ATP5J, DECR1 as discussed above, as well as Coiled-Coil-Helix-Coiled-Coil-Helix Domain Containing 10 (CHCHD10), a mitochondrial protein that may play a role in oxidative phosphorylation,[18] and cysteine and glycine rich protein 3 (CSRP3), a muscle LIM domain protein. Representative data for known biomarkers and several novel proteins are demonstrated in Figure 1.

In additional exploratory analysis, we also compared SMI versus control samples without filtering for proteins that were changed in PMI. This analysis yielded an additional 58 proteins elevated in SMI vs. the control cohort, (Supplemental Table 2), which included the “rediscovery” of IL1RL1(ST2), as well as many novel proteins including the mitochondrial iron-sulfur cluster assembly factor BolA family member 3 (BOLA3) and the chloride intracellular channel 4 (CLIC4).

Technical validation by mass spectrometry

To rigorously verify the specificity of observed changes in several novel putative biomarkers including ADSSL1, olfactomedin like 3 (OLFML3) and glucose-6-phosphate isomerase (GPI), we performed technical validation studies using orthogonal mass spectrometry based techniques as detailed in Supplemental Methods. The representative data in Supplemental Figure 2 using aptamer enriched-multiple reaction monitoring (MRM) demonstrates a striking concordance with findings from the expanded aptamer platform and provides accurate quantitation of our protein findings. Supplemental Table 5 details the peptide sequences, precursor masses and transition ions selected for MRM analysis.

DISCUSSION

We previously reported the application of a 1,129-plex aptamer proteomic assay to individuals undergoing planned myocardial injury.[1] Here we markedly expanded the scope of prior investigations. While testing the feasibility of large scale multiplexing with a platform that now measures ~5,000 analytes, we provide novel biological insights into the response to injury in humans. Concordant with the ~4-fold increased breadth of the platform, we identified ~ 3-fold number of new protein changes. It is notable that greater than 75% of our prior aptamer findings[1] were recapitulated in this study with concordant directionality ($P < 0.05$), though here we studied more catheterization controls and excluded many additional proteins that appear to be influenced by heparin treatment. Further, twenty-two of the proteins described in two recent mass spectrometry based proteomic profiling papers were confirmed using this new technique;[9, 10] an additional ~150 novel changes were identified in the present investigation.

As compared to the prior iteration of the platform, this version covers a broader array of intracellular proteins (approximately 30%), many of which are reproducibly identified in human plasma and changed in the setting of myocardial injury. We were interested to find an enrichment of these proteins following myocardial injury, as 19 of the 33 (58%) most significantly changed proteins are intracellular. Amongst the novel markers identified in both planned and spontaneous injury, we found many proteins, including CHCHD10, DECR1, and MDH1and MDH2, suggesting the release of mitochondrial proteins early after myocardial injury. CSRP3, which was increased after SMI and PMI, is hypothesized to mediate cardiac mechanosensation[19] and has been associated with familial and idiopathic dilated and hypertrophic cardiomyopathy.[20]

In addition to identifying low abundance intracellular proteins not previously assayed in the blood, the expanded platform also provides insight into biologically active proteins which are released following myocardial injury. For example, adenylosuccinate synthetase 1 (ADSSL1) is found in cardiac and skeletal muscle and demonstrates greater than 14-fold increase in circulating levels within 1 hour after myocardial injury. It is a muscle-specific enzyme that plays a role in purine nucleotide cycle by catalyzing the first step in the conversion of inosine monophosphate to adenosine monophosphate, and mutations in the gene have been reportedly associated with myopathy.[21] Of particular interest are proteins that may have hormone-like functions on other tissues. The mitochondrial ATP synthase-coupling factor 6 (ATP5J), the most substantially increased protein (by 10 minutes) following myocardial injury, is reported to be a vasoactive peptide. It binds to plasma membrane ATP synthase, leading to vasoconstriction, intracellular acidosis, inhibition of prostacyclin and nitric oxide generation resulting in blood pressure elevation.[22]

Our study has several limitations. While this expanded aptamer-based proteomics platform provides very broad coverage with high throughput, it is agnostic to changes of analytes not targeted. Exclusion of nearly 2000 proteins that changed in the catheterization control samples translates into a very conservative effort to limit potential confounding factors, possibly excluding proteins that may be changed in myocardial injury as well as catheterization. Additionally, because groups of proteins cluster within biological pathways,

the assumption of independent statistical tests used in our Bonferroni-corrected P values is overly stringent, increasing the possibility of false negative results. Similarly, while we have emphasized characteristics of individual markers in our derivation and validation analyses, we anticipate that a multi-marker approach might ultimately be used clinically. Of note, we evaluated temporal co-variance as a method to condense the number of proteins for diagnostic value. In a pilot analysis that focused on proteins that were significantly elevated at one or more time points from baseline following PMI, we identified six temporal protein groupings (Supplemental Figure 3). Carrying forward a subset of proteins with the largest effect size from these groupings (excluding Troponin I and CK-MB), we found 39 principal components that accounted for 85% of the variance in the SMI group versus controls. This approach modestly separated the groups with $P = 0.00077$ (Supplemental Figure 4). One might hypothesize that specific markers would be most useful when patients are stratified by time to presentation, though this would necessitate future studies in larger numbers of patients. Finally, given that the total number of patients in our cohorts is relatively small, additional samples will also be necessary to identify markers specific to hypertrophic cardiomyopathy patients or proteins that are associated with specific medications or clinical traits.

In summary, we demonstrate the feasibility of large scale aptamer multiplexing at a scale that has not previously been reported. Further, sample throughput of this platform allows one to assess more than 4800 proteins in hundreds of samples within a week, greatly exceeding the sample throughput approachable by other strategies. To provide additional support of these proof-of-concept findings, efforts are ongoing aimed at both additional analytical validation using orthogonal techniques as well as clinical validation in large, heterogeneous cohorts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

JJ, DN, LF, MDB, TM, LLJ and REG contributed to study design. JJ, DN, SG, MK, TM, LLJ, and REG analyzed and interpreted the data. NF and RP conducted mass spectrometry experiments, acquired and analyzed data. JJ, DN, RP, LLJ and REG wrote the manuscript.

SOURCES OF FUNDING: These studies were supported by NIH grants HHSN268201000033C, R01HL132320 and R01HL133870 to REG, T32HL007208 to MDB and K01GM103817 to DN.

References

1. Anderson NL, Polanski M, Pieper R, Gatlin T, Tirumalai RS, Conrads TP, Veenstra TD, Adkins JN, Pounds JG, Fagan R, Lobley A. The human plasma proteome: A nonredundant list developed by combination of four separate sources. Mol Cell Proteomics. 2004; 3:311–326. [PubMed: 14718574]
2. Fagerberg L, Oksvold P, Skogs M, Algenas C, Lundberg E, Ponten F, Sivertsson A, Odeberg J, Klevebring D, Kampf C, Asplund A, Sjostedt E, Al-Khalili Szigyarto C, Edqvist PH, Olsson I, Rydberg U, Hudson P, Ottosson Takanen J, Berling H, Bjorling L, Tegel H, Rockberg J, Nilsson P, Navani S, Jirstrom K, Mulder J, Schwenk JM, Zwahlen M, Hober S, Forsberg M, von Feilitzen K, Uhlen M. Contribution of antibody-based protein profiling to the human chromosome-centric proteome project (c-hpp). J Proteome Res. 2013; 12:2439–2448. [PubMed: 23276153]

3. Ping P, Vondriska TM, Creighton CJ, Gandhi TK, Yang Z, Menon R, Kwon MS, Cho SY, Drwal G, Kellmann M, Peri S, Suresh S, Gronborg M, Molina H, Chaerkady R, Rekha B, Shet AS, Gerszten RE, Wu H, Raftery M, Wasinger V, Schulz-Knappe P, Hanash SM, Paik YK, Hancock WS, States DJ, Omenn GS, Pandey A. A functional annotation of subproteomes in human plasma. Proteomics. 2005; 5:3506–3519. [PubMed: 16104058]

4. Gerszten RE, Accurso F, Bernard GR, Caprioli RM, Klee EW, Klee GG, Kullo I, Laguna TA, Roth FP, Sabatine M, Srinivas P, Wang TJ, Ware LB. Challenges in translating plasma proteomics from bench to bedside: Update from the nhlbi clinical proteomics programs. Am J Physiol Lung Cell Mol Physiol. 2008; 295:L16–22. [PubMed: 18456800]

5. Juncker D, Bergeron S, Laforte V, Li H. Cross-reactivity in antibody microarrays and multiplexed sandwich assays: Shedding light on the dark side of multiplexing. Curr Opin Chem Biol. 2014; 18:29–37. [PubMed: 24534750]

6. Ngo D, Sinha S, Shen D, Kuhn EW, Keyes MJ, Shi X, Benson MD, O'Sullivan JF, Keshishian H, Farrell LA, Fifer MA, Vasan RS, Sabatine MS, Larson MG, Carr SA, Wang TJ, Gerszten RE. Aptamer-based proteomic profiling reveals novel candidate biomarkers and pathways in cardiovascular disease. Circulation. 2016; 134:270–285. [PubMed: 27444932]

7. Lakkis NM, Nagueh SF, Dunn JK, Killip D, Spencer WH 3rd. Nonsurgical septal reduction therapy for hypertrophic obstructive cardiomyopathy: One-year follow-up. J Am Coll Cardiol. 2000; 36:852–855. [PubMed: 10987610]

8. Lakkis NM, Nagueh SF, Kleiman NS, Killip D, He ZX, Verani MS, Roberts R, Spencer WH 3rd. Echocardiography-guided ethanol septal reduction for hypertrophic obstructive cardiomyopathy. Circulation. 1998; 98:1750–1755. [PubMed: 9788829]

9. Keshishian H, Burgess MW, Gillette MA, Mertins P, Clauser KR, Mani DR, Kuhn EW, Farrell LA, Gerszten RE, Carr SA. Multiplexed, quantitative workflow for sensitive biomarker discovery in plasma yields novel candidates for early myocardial injury. Molecular & cellular proteomics : MCP. 2015; 14:2375–2393. [PubMed: 25724909]

10. Addona TA, Shi X, Keshishian H, Mani DR, Burgess M, Gillette MA, Clauser KR, Shen D, Lewis GD, Farrell LA, Fifer MA, Sabatine MS, Gerszten RE, Carr SA. A pipeline that integrates the discovery and verification of plasma protein biomarkers reveals candidate markers for cardiovascular disease. Nature biotechnology. 2011; 29:635–643.

11. Keshishian H, Addona T, Burgess M, Mani DR, Shi X, Kuhn E, Sabatine MS, Gerszten RE, Carr SA. Quantification of cardiovascular biomarkers in patient plasma by targeted mass spectrometry and stable isotope dilution. Mol Cell Proteomics. 2009; 8:2339–2349. [PubMed: 19596694]

12. Lewis GD, Farrell L, Wood MJ, Martinovic M, Arany Z, Rowe GC, Souza A, Cheng S, McCabe EL, Yang E, Shi X, Deo R, Roth FP, Asnani A, Rhee EP, Systrom DM, Semigran MJ, Vasan RS, Carr SA, Wang TJ, Sabatine MS, Clish CB, Gerszten RE. Metabolic signatures of exercise in human plasma. Sci Transl Med. 2010; 2:33ra37.

13. Okamoto F, Sohmiya K, Ohkaru Y, Kawamura K, Asayama K, Kimura H, Nishimura S, Ishii H, Sunahara N, Tanaka T. Human heart-type cytoplasmic fatty acid-binding protein (h-fabp) for the diagnosis of acute myocardial infarction. Clinical evaluation of h-fabp in comparison with myoglobin and creatine kinase isoenzyme mb. Clinical chemistry and laboratory medicine : CCLM / FESCC. 2000; 38:231–238.

14. Glatz JF, van der Vusse GJ, Simoons ML, Kragten JA, van Dieijen-Visser MP, Hermens WT. Fatty acid-binding protein and the early detection of acute myocardial infarction. Clinica chimica acta; international journal of clinical chemistry. 1998; 272:87–92. [PubMed: 9581860]

15. Collinson IR, Runswick MJ, Buchanan SK, Fearnley IM, Skehel JM, van Raaij MJ, Griffiths DE, Walker JE. Fo membrane domain of atp synthase from bovine heart mitochondria: Purification, subunit composition, and reconstitution with f1-atpase. Biochemistry. 1994; 33:7971–7978. [PubMed: 8011660]

16. Fillgrove KL, Anderson VE. The mechanism of dienoyl-coa reduction by 2,4-dienoyl-coa reductase is stepwise: Observation of a dienolate intermediate. Biochemistry. 2001; 40:12412–12421. [PubMed: 11591162]

17. Sun H, Li N, Wang X, Chen T, Shi L, Zhang L, Wang J, Wan T, Cao X. Molecular cloning and characterization of a novel muscle adenylosuccinate synthetase, adssl1, from human bone marrow stromal cells. Mol Cell Biochem. 2005; 269:85–94. [PubMed: 15786719]

18. Martherus RS, Sluiter W, Timmer ED, VanHerle SJ, Smeets HJ, Ayoubi TA. Functional annotation of heart enriched mitochondrial genes gbas and chchd10 through guilt by association. Biochem Biophys Res Commun. 2010; 402:203–208. [PubMed: 20888800]

19. Wang X, Li Q, Adhikari N, Hall JL. A role for muscle lim protein (mlp) in vascular remodeling. Journal of molecular and cellular cardiology. 2006; 40:503–509. [PubMed: 16519896]

20. Geier C, Gehmlich K, Ehler E, Hassfeld S, Perrot A, Hayess K, Cardim N, Wenzel K, Erdmann B, Krackhardt F, Posch MG, Osterziel KJ, Bublak A, Nagele H, Scheffold T, Dietz R, Chien KR, Spuler S, Furst DO, Nurnberg P, Ozcelik C. Beyond the sarcomere: Csrp3 mutations cause hypertrophic cardiomyopathy. Hum Mol Genet. 2008; 17:2753–2765. [PubMed: 18505755]

21. Wen HY, Xia Y, Young ME, Taegtmeyer H, Kellems RE. The adenylosuccinate synthetase-1 gene is activated in the hypertrophied heart. J Cell Mol Med. 2002; 6:235–243. [PubMed: 12169208]

22. Osanai T, Mikami K, Kitajima M, Urushizaka M, Kawasaki K, Tomisawa T, Itaki C, Noto Y, Magota K, Tomita H. Nutritional regulation of coupling factor 6, a novel vasoactive and proatherogenic peptide. Nutrition. 2017; 37:74–78. [PubMed: 28359367]

Clinical Perspective

What is new?

- Like antibodies, DNA aptamers can be generated as affinity reagents for proteins.
- Emerging data suggest that they can be used to measure blood protein levels in clinical cohorts; however the technology remains in its infancy.
- Here we tested the scalability of this approach with a markedly expanded platform containing ~ 5,000 aptamers targeting a far broader range of analytes than previously examined using this technology.
- We applied the platform to a cohort of individuals undergoing “planned myocardial injury” (PMI) for hypertrophic cardiomyopathy.
- In addition to confirming findings from prior studies, we identified nearly 150 additional putative markers of myocardial injury.

What are the clinical applications?

- Our study suggests that the platform can be applied to identify very early markers of myocardial injury.
- Such markers might hasten the diagnosis of myocardial injury, as well as the administration of appropriate therapy.
- Our data also provide new insights into the human response to injury as well as new potential targets for therapeutic intervention.
- Finally, the improvements in breadth and throughput of this new platform suggest that it can be applied to large patient cohorts to address a variety of important clinical questions.

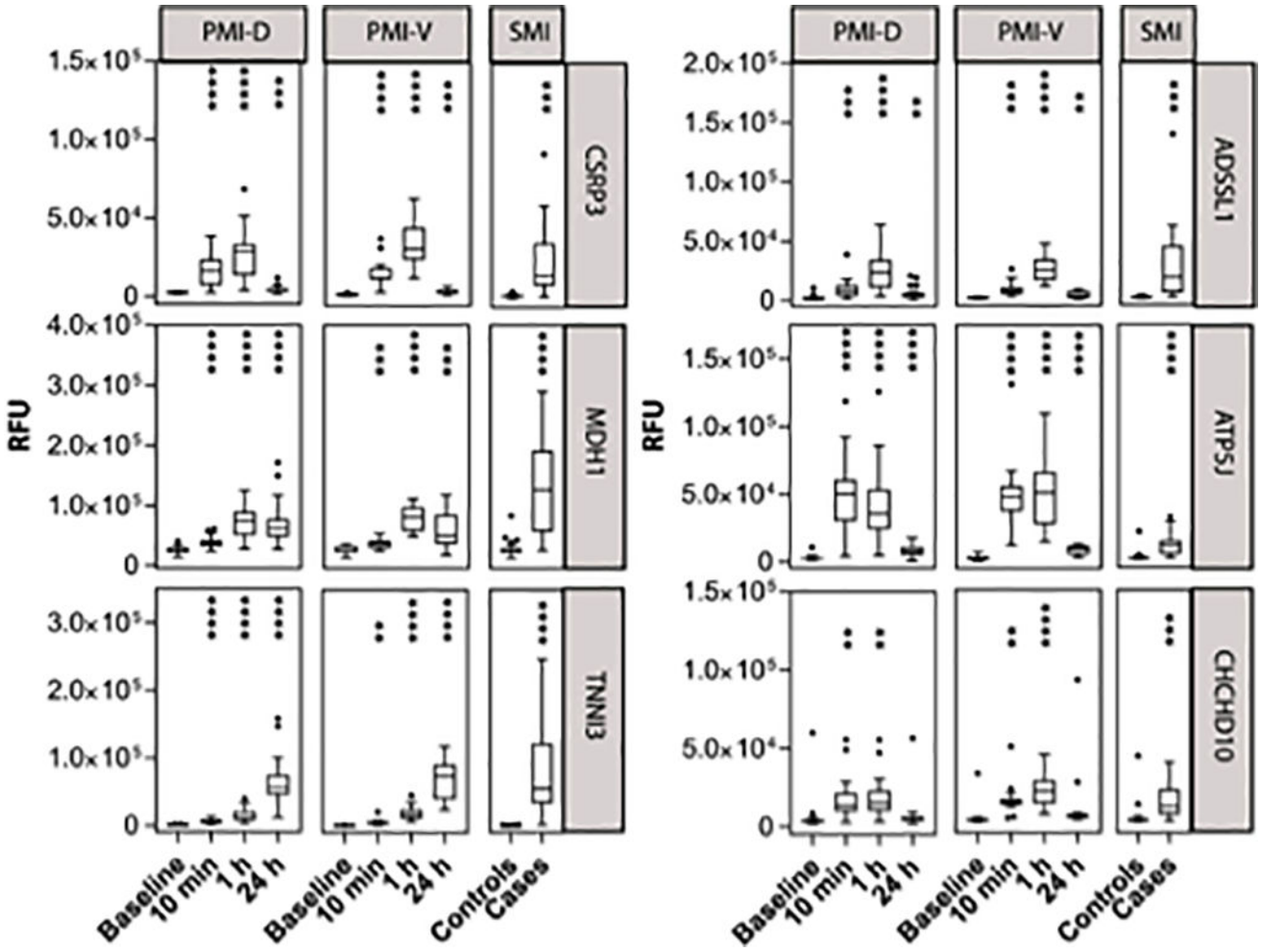

Figure 1. Protein markers that are increased early after the onset of planned myocardial injury (PMI) and in spontaneous myocardial injury (SMI)

Data presented are from PMI derivation (PMI-D) and validation (PMI-V) cohorts of selected proteins that increased in both a) derivation (n=20) and validation (n=15) cohorts (P< 1.05E-05) and b) SMI cohorts (n=63) P < 6.17–04. P values calculated by one-way repeated measures ANOVA performed on log transformed RFU values. Edges of boxes denote 25th and 75th percentiles, lines denote median, and whiskers are plotted using Tukey method. **** P < 0.0001, *** P <0.001, ** P < 0.01; where P represents significance of change from baseline.

Table 1

Top protein changes in spontaneous MI

Entrez Symbol	UniProt ID	Target Protein Name	Median Fold Change	*P*
TNNI	P19429	troponin I, cardiac	66.7	5.93E-16
ADSSL1	Q8N142	adenylosuccinate synthase like 1	15.3	1.73E-12
CKB, CKM	P12277, P06732	creatine kinase M-type: B-type heterodimer	10	1.80E-13
FABP3	P05413	fatty acid binding protein 3, muscle and heart	8.3	3.69E-11
CSRP3	P50461	cysteine and glycine-rich protein 3	6.36	2.55E-11
ATP5J	P18859	ATP synthase, mitochondrial Fo complex, subunit F6	5.27	5.30E-11
MDH1	P40925	malate dehydrogenase, cytoplasmic	5.2	4.20E-12
CKM	P06732	creatine kinase M-type	3.99	1.18E-11
CHCHD10	Q8WYQ3	coiled-coil-helix-coiled-coil-helix domain containing 10	3.78	4.01E-09
MDH2	P40926	malate dehydrogenase 2, NAD (mitochondrial)	3.19	4.59E-09
TPM3	P06753	tropomyosin 3	2.93	7.06E-07
REXO2	Q9Y3B8	REX2, RNA exonuclease 2 homolog	2.34	1.01E-08
DUSP3	P51452	dual specificity phosphatase 3	2.08	4.31E-05
HIST2H2BE	Q16778	histone cluster 2, H2be	2.03	1.66E-04
LANCL1	O43813	LanC lantibiotic synthetase component C-like 1	2.03	1.83E-06
TPI1	P60174	triosephosphate isomerase 1	2.01	3.16E-06
HDHD2	Q9H0R4	haloacid dehalogenase-like hydrolase domain 2	1.94	1.08E-07
MB	P02144	myoglobin	1.94	3.87E-07
CRIP2	P52943	cysteine-rich protein 2	1.89	1.07E-07
TKT	P29401	transketolase	1.78	6.40E-07
PEBP1	P30086	phosphatidylethanolamine-binding protein 1	1.66	6.35E-06
RPS6KA1	Q15418	ribosomal protein S6 kinase, 90kDa, polypeptide 1	1.66	2.34E-05
FCN1	O00602	ficolin (collagen/fibrinogen domain containing) 1	1.62	3.19E-05
PCDHGB1	Q9Y5G3	protocadherin gamma subfamily B, 1	1.61	2.07E-07
GLRX2	Q9NS18	glutaredoxin 2	1.55	1.47E-08
DECR1	Q16698	2,4-dienoyl CoA reductase 1, mitochondrial	1.51	4.64E-04
LST1	O00453	leukocyte specific transcript 1	1.45	3.87E-07
NAP1L2	Q9ULW6	nucleosome assembly protein 1-like 2	1.27	4.10E-04
SUMF2	Q8NBJ7	sulfatase modifying factor 2	1.24	5.05E-05

Shown are proteins increased in SMI cases vs. controls with P <6.17E-04, listed in descending fold change from control (Wilcoxon rank-sum on log transformed RFU values). All proteins were also significant and increased within 1 hour after injury in both PMI derivation and validation cohorts (P < 1.05E-05 and P < 1.33E-04, respectively).

Table 2

Top proteins increased in the peripheral blood after myocardial injury

Entrez Symbol	UniProt ID	Target Protein Name	Derivation, n=20, Median % Change				Validation, n=15 Median % Change			
			10 min vs. Baseline	1 h vs. Baseline	24 h vs. Baseline	*P*	10 min vs. Baseline	1 h vs. Baseline	24 h vs. Baseline	*P*
ADSSL1	Q8N142	adenylosuccinate synthase like 1	437.6	1407.2	108.1	8.64E-10	473.2	1952.1	122.2	2.81E-09
ARHGAP1	Q07960	Rho GTPase activating protein 1	49.9	48	5.1	2.24E-07	49.2	51.1	1.7	1.02E-10
ATP5J	P18859	ATP synthase, mitochondrial Fo complex, subunit F6	1492.2	1044	215.9	1.41E-09	1913.2	1693.6	220.4	6.58E-20
C1orf185	Q5T7R7	chromosome 1 open reading frame 185	40.9	55.1	8.9	2.36E-07	44	45.5	13.7	1.11E-10
CHCHD10	Q8WYQ3	coiled-coil-helix-coiled-coil-helix domain containing10	258.7	271.4	41.4	3.41E-06	310.1	423.9	47.8	6.05E-06
CKB,CKM	P12277 P06732	creatinine kinase, brain/muscle-type heterodimer	117.4	326.9	389.6	1.19E-08	122.6	475	275.7	9.12E-08
CKM	P06732	creatine kinase, muscle	31.7	124.9	186.8	6.24E-09	35.7	163.8	224.6	4.95E-08
COL23A1	Q86Y22	collagen, type XXIII, alpha 1	51.9	48.7	−1.3	2.33E-10	50.1	40.2	−2.8	4.34E-16
CRABP2	P29373	cellular retinoic acid binding protein 2	62.3	64.8	16.8	1.08E-06	64.1	67.3	7.5	5.62E-18
CRIP2	P52943	cysteine-rich protein 2	45.3	74.1	−7.4	3.12E-06	38.3	74.2	−10.3	2.34E-05
CSRP3	P50461	cysteine and glycine-rich protein 3	496.5	841.5	65.5	1.82E-10	492.9	1179.8	71	5.46E-22
DAG1	Q14118	dystrophin-associated glycoprotein 1	43.4	25.9	−9.4	2.71E-06	55.6	35.5	−0.2	1.95E-08
DDR1	Q08345	discoidin domain receptor tyrosine kinase 1	55	84.1	15.4	5.03E-06	53.4	67	25.5	6.73E-07
DECR1	Q16698	2,4-dienoyl CoA reductase 1, mitochondrial	568.6	584.4	−0.6	6.63E-07	779.2	781.6	−26.5	3.95E-18
FABP3	P05413	fatty acid binding protein 3, muscle and heart	127.4	439.3	35.3	3.18E-21	147.7	536.6	37.6	7.84E-18
FCN1	O00602	ficolin (collagen/fibrinogen domain containing) 1	31.8	40.9	13.6	6.60E-08	30.5	37	12.7	5.02E-06
FKBP2	P26885	FK506 binding protein 2, 13kDa	39.3	40.5	8.7	2.60E-07	48.6	43.9	17.3	1.23E-10
GLRX2	Q9NS18	glutaredoxin 2	34.4	51.3	12.4	9.34E-06	38.6	91.1	17.1	4.02E-06
H1FX	Q92522	H1 histone family, member X	772.6	457.8	54.5	7.11E-08	1004.2	926.7	25	1.63E-19
MAZ	P56270	MYC-associated zinc finger protein	35.2	50.7	18.3	1.57E-06	47	45.5	15.3	1.30E-07
MB	P02144	myoglobin	52.9	113.7	37.8	9.24E-11	51.8	135.9	43.6	4.45E-08
MDH1	P40925	malate dehydrogenase 1, NAD (soluble)	43.7	170.7	132.7	2.30E-11	43.8	186.4	99.6	9.87E-09
MDH2	P40926	malate dehydrogenase 2, NAD (mitochondrial)	551.8	1037.7	211.7	9.61E-11	893.5	1652.7	173.7	4.60E-08
NDUFV2	P19404	NADH dehydrogenase (ubiquinone) flavoprotein 2	315	422.5	52	2.02E-08	406.2	700.3	57.8	8.01E-06
OLFML3	Q9NRN5	olfactomedin-like 3	36.4	−3.4	−24.9	5.87E-10	34.7	−7.8	−19.2	4.69E-06
PCDHGB1	Q9Y5G3	protocadherin gamma subfamily B, 1	71.6	79.2	25.8	1.90E-09	81	70.2	15.9	1.62E-10

Entrez Symbol	UniProt ID	Target Protein Name	Derivation, n=20, Median % Change				Validation, n=15 Median % Change			
			10 min vs. Baseline	1 h vs. Baseline	24 h vs. Baseline	*P*	10 min vs. Baseline	1 h vs. Baseline	24 h vs. Baseline	*P*
PEBP1	P30086	phosphatidylethanolamine binding protein 1	33.6	72.9	−9.4	5.24E-06	36.6	78.2	−12	8.84E-11
PKLR	P30613	pyruvate kinase, liver and RBC	59.7	49.1	33.6	9.83E-07	53.5	73.2	17.8	1.63E-10
REXO2	Q9Y3B8	REX2, RNA exonuclease 2 homolog	31.7	53.5	54.4	5.08E-07	32.5	79.9	32.6	3.96E-09
TKT	P29401	transketolase	55.4	79.5	11.4	6.04E-07	34.7	65.2	−0.5	3.14E-10
TNNI3	P19429	troponin I type 3 (cardiac)	347.7	909.8	4614.8	2.02E-13	361.6	1279.9	5926.6	3.36E-12
TPI1	P60174	triosephosphate isomerase 1	54.1	87.7	14.6	4.96E-07	30.6	97.5	0.7	4.22E-12

Proteins found to be increased by greater than 30% within 10 minutes after PMI in both derivation and validation cohorts (global $P < 1.05E-05$ and $P < 1.33E-04$, respectively, for change within 24-hour period by one-way repeated measures ANOVA on log transformed RFU values). Change values denote median percent change. Proteins listed in alphabetical order.